RAYONG OIL SPILL CLEANUP WORKERS
EXPOSURE AND SYMPTOM ASSESSMENT

by

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ABSTRACT

In July of 2013, a pipeline connecting an offshore oil platform to a tanker, operated by PTT Global Chemical (PTTGC), a corporation owned by the government of Thailand, leaked and caused crude oil to spill into the Sea of Rayong off the coast of Thailand. The crude oil covered an area of approximately 20 square kilometers and washed ashore on the island of Samet in an area called "Ao Prao" on 28 July, 2013. On-land cleanup lasted about a month and was performed by a combination of territorial defense volunteers, citizen volunteers, Thai military personnel and PTTGC employees. Cleanup procedures included oil containment and dispersal using absorbent pads, and removal and disposal of contaminated soil, sand and rocks. The goal of this dissertation is to determine if Rayong oil spill cleanup workers were exposed to elevated levels of PAHs and benzene and if these exposures are associated with recorded acute symptoms.

We measured the concentration of 1-hydroxypyrene-glucuronide (1-OHPG), a metabolite of pyrene, in the 1,343 frozen stored urine samples available from the cleanup workers, and retrieved previously measured trans,trans-muconic acid (t,t-MA) data, a benzene metabolite. This allowed us to quantify the internal dose of polycyclic aromatic hydrocarbons (PAHs) and benzene in these workers and to examine factors related to their dose. During the early days of cleanup, urinary 1-OHPG of the workers was elevated, comparable to occupational exposures, and declined to near background (general population) levels in workers by the end of the cleanup operation. This was consistent with our hypothesis that the exposure levels of PAHs would be the highest in the first week of cleanup and decline thereafter. Detectable levels of t,t-MA also exhibited a decreasing trend over the course of the cleanup period. Job descriptions with the highest levels of urinary 1-OHPG after adjustment were oil dispersant applicators and contaminated sand/trash handlers.

Prevalence of several post-shift symptoms, including irritation of throat and nose, increased with concentration of urinary 1-OHPG. Similarly, one group of symptoms determined by factor analysis, designated as "irritative symptoms", including irritation of the eye, throat and/or nose,

eye injection (redness) and excessive tearing (epiphora) was associated with increased concentration of urinary 1-OHPG.

In conclusion, Rayong oil spill cleanup workers exhibited evidence of elevated levels of PAH and benzene exposure during the early weeks of cleanup, compared to near background levels 4 weeks after cleanup began. These workers also demonstrated an association between prevalence of acute irritative symptoms and PAH exposure measured by urinary 1-OHPG. Long-term health monitoring of oil spill cleanup workers should be implemented, particularly among those workers suspected of sustaining high exposure to crude oil.

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PREFACE

This dissertation consists of five chapters. The first chapter is an introduction that summarizes the Rayong oil spill incident, the subsequent cleanup efforts, and the specific aims of this dissertation. Chapter 2 presents the rational for the dissertation based on literature reviews of general oil spill cleanup procedures and research, the Rayong oil spill details, the composition of crude oil, potential toxicants (PAHs and benzene) and confounding factors. Chapters 3 and 4 are organized and presented in manuscript format. The first manuscript (Chapter 3) describes our study to quantify internal dose of PAHs and benzene in the Rayong oil spill cleanup workers and to examine factors related to their internal dose. In the second manuscript (Chapter 4), we examined prevalence of post-shift acute symptoms among Rayong oil spill cleanup workers, and assessed their association with predictive factors, including internal dose biomarkers of PAH and benzene exposure, day of cleanup worked, job description, PPE use, and age of workers. Lastly, Chapter 5 summarizes our research conclusions according to the specific aims, public health implications, and suggestions for future research.

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ABBREVIATIONS

1-OHPG	1-Hydroxypyrene-Glucuronide
8-OHdG	8-Hydroxy-2'–Deoxyguanosine
ADH	Alcohol Dehydrogenase
ALDH	Aldehyde Dehydrogenase
ALT	Alanine Aminotransferase
AML	Acute Myelogenous Leukemia
ANLL	Acute Nonlymphocytic Leukemia
BP Oil Spill	Deepwater Horizon Oil Spill
CBC	Complete Blood Count
CES-D	Center for Epidemiologic Studies Depression
COX	Cyclooxygenase
CYP	Cytochrome P450
DHDD	Dihydrodiol Dehydrogenase
DNA	Deoxyribonucleic Acid
EH	Epoxide Hydrolase
EFA	Exploratory Factor Analysis
EROD	Ethoxyresorufin-O-de-ethylase
FEF	Forced Expiratory Flow
FEV1	Forced Expiratory Volume in the First Second
FVC	Forced Vital Capacity
GAD	Generalized Anxiety Disorder
GC	Gas Chromatography
GEE	Generalized Estimating Equation
GSH	Glutathione
HPLC	High-Performance Liquid Chromatography

IARC	International Agency for Research on Cancer
IQR	Interquartile Range
LOX	Lipoxygenase
LTB	Leukotriene B
MDA	Malondialdehyde
MN	Micronucleus
MPO	Myeloperoxidase
MR	Minimal Residuals
NQO1	NAD(P)H Quinone Oxidoreductase
OSHA	Occupational Safety and Health Administration
PAH	Polycyclic Aromatic Hydrocarbon
PBS	Phosphate Buffered NaCl
PDS	Posttraumatic Diagnostic Scale
PEF	Peak Expiratory Flow
PG	Prostaglandine
PM-10	Particulate Matter 10 Micrometers or Less in Diameter
PPE	Personal Protective Equipment
PWI	Psychological Well-Being Index
SCL-36	Symptom Checklist-36
SCL-90-R	Symptom Checklist-90-Revision
SGPT	Serum Glutamic-Pyruvic Transaminase
STAI-X-1	State-Trait Anxiety Inventory
t,t-MA	trans,trans-Muconic Acid
UME	Unusual Mortality Events
USD	United States Dollar (US Dollar)
VOC	Volatile Organic Compound

CHAPTER 1: INTRODUCTION

On 27 July, 2013, the pipeline connecting an offshore oil platform to a tanker, operated by PTT Global Chemical (PTTGC), a corporation owned by the government of Thailand, leaked and caused crude oil to spill into the sea of Rayong off the coast of Thailand. The crude oil covered an area of approximately 20 square kilometers and washed ashore on the island of Samet in an area called "Ao Prao" on 29-31 July, 2013.[1, 2] Although PTTGC estimated that the amount of oil spilled was about 50 cubic meters or 336 barrels,[2] other experts believe the true volume to be as high as 190 cubic meters or 1,200 barrels.[3]

Clean-up procedures began at Ao Prao on 29 July, 2013 and ended a month later on 30 August, 2013.[1] These procedures included containing, skimming, and dispersing the oil slick, using absorbent pads on land, and digging up and disposing of contaminated soil, sand and rocks. The on-land cleanup was performed by a combination of territorial defense volunteers, citizen volunteers, military soldiers and PTTGC workers.[3]

Crude oil is a complex mixture of hydrocarbons, some of which may affect human health, including volatile organic compounds (VOCs), such as benzene and naphthalene, and polycyclic aromatic hydrocarbons (PAHs), such as pyrene and benzo[a]pyrene.[4] The International Agency for Research on Cancer (IARC) has classified benzene and benzo[a]pyrene as group 1 carcinogens, [5] known to cause cancer in humans.

The Rayong Provincial Public Health Office and Rayong Hospital designed a health surveillance plan for the workers, collecting urine samples post-shift to assess urinary tran,tran-muconic acid (t,t-MA), a metabolite of benzene. However, given the lack of smoking status[6] data and confounding effects of smoking on urinary t,t-MA concentration,[7] we believe these exposure estimates should be re-examined and expanded to include estimates of PAH exposure and a tobacco biomarker (cotinine).[8]

In addition, acute symptoms including respiratory symptoms and irritation symptoms were observed and recorded in health survey questionnaires administered after worker's shifts – even in

workers with urinary t,t-MA levels below 500 ug/gCr (OSHA Standard).[9] About 1,343 left-over urine samples from the Rayong Hospital's analysis were stored at -30° Celsius as part of the original surveillance plan, however, government funding was suspended and there was no plan for further use of these samples. Subsequently, the urine samples, packed in dry-ice were shipped to Dr. Strickland's lab in Baltimore for laboratory analysis, including measuring the PAH metabolite, 1-hydroxypyrene-glucuronide (1-OHPG), as well as creatinine and cotinine, to expand our understanding of the exposures sustained by these workers and lay the groundwork for further assessment of potential acute and chronic health effects.

Specific Aims

The goal of this dissertation is to determine if Rayong oil spill cleanup workers were exposed to elevated levels of PAHs and benzene and if these exposures are associated with recorded acute symptoms.

The specific aims of this study are the following:

Aim 1: To determine the internal dose of PAHs and benzene among 1,343 Rayong oil spill cleanup workers by measuring the PAH biomarker 1-OHPG in previously collected urine samples and re-examining the urinary t,t-MA concentrations previously measured.

Aim 2: To compare the internal dose of workers who worked on different days of cleanup: early week 1 (day 2-4), late week 1 (day 5-7), week 2, week 3 and week 4, adjusting for smoking status (cotinine) and to examine factors related to their dose, including personal protective equipment (PPE) use and job descriptions.

Aim 3: To examine the association between levels of internal dose biomarkers measured in the workers and acute symptoms previously recorded.

These aims were achieved by measuring an internal dose biomarker of PAHs (1-OHPG) and a biomarker of tobacco smoking (cotinine) in the 1,343 frozen urine samples, combined with information from the questionnaires and t,t-MA measurements previously recorded. The questionnaire contained data on demographic factors, cleanup jobs, days of cleanup worked, PPE use, smoking status (partial data) and symptoms.

The main **hypothesis** of our study is that workers who worked during the early days of cleanup will have higher levels of exposure and higher prevalence of acute symptoms than those that worked in subsequent weeks when exposure was reduced by the cleanup process and natural degradation.

CHAPTER 2: BACKGROUND

A. General Oil Spill Background and Research

Oil spills involve the release or discharge of a liquid petroleum hydrocarbon into the environment, either on land or offshore.[1] The size and frequency of oil spills has increased with the global development of crude oil production. Oil spills are caused by several common factors: human error, instrument error, or natural disaster.

Oil spills can be caused by human mistakes or carelessness. An example was the spill incident of the Atlantic Empress when the oil tanker collided with Aegean Captain, another supertanker off the coast of Liberia.[2] In Alaska, the Exxon Valdez oil spill was another example, where the ship collided with a reef while the captain, reported to have been drinking heavily the night of the incident, was not at the controls.[3]

Oil spills can be caused by instrument or equipment failures. Malfunction of instruments related to an oil platform including pipelines, mechanical valves and a blowout preventer were the cause of the Deepwater Horizon (BP) oil spill. An explosion on the oil platform followed by the failure of the main blowout preventer was responsible for this massive spill.[4] The Rayong oil spill, the focus of my dissertation, was an example of pipeline leakage.[1]

Oil spills can also be related to natural disasters. Such was the case in the Prestige tanker oil spill. One of the fuel tanks of the Prestige tanker exploded due to a storm and the tanker sank in the gulf of Galicia, Spain.[5]

1. Size or Tiers of Oil Spills

"Tier levels" are used to describe the size and scope of a potential oil spill response. The tiered approach to oil spill planning and preparedness is used by U.S. and International governmental and non-governmental organizations in developing oil spill response strategies, response team structures, and training and exercise programs. Note that tier levels are typically not associated with the volume of oil spilled. It is the overall impact of the spill, not the quantity alone, that dictates the types and amounts of resources required and duration of cleanup operations.[6-8] To illustrate, 20 cubic meters of crude oil spilled in a small canal might be considered more severe than the same amount spilled in the Pacific Ocean. Thus, the size of the contaminated marine area or the concentration of the spilled crude oil must also be considered. It is recommended that organizations adopt a 3-tier oil spill response planning system, similar to the example below, in order to scale oil spill response training and exercise programs in an efficient manner.[6-8]

Tier 1: Minor spills, including incipient spills that are quickly controlled, contained and cleaned up using local (onsite or immediately available) equipment and personnel resources. A Tier 1 spill would typically be resolved within a few hours or days. The approximate spilled volume is usually up to about 20 cubic meters.

Tier 2: Moderate spills requiring activation of significant regional oil spill response resources. A Tier 2 spill response may continue for several days or weeks. The approximate spilled volume is roughly between 20 to 1,000 cubic meters.

Tier 3: Major spills requiring activation of large quantities and multiple types of response resources including those from out of the region, and possibly international sources. A Tier 3 spill response may continue for many weeks or months. The approximate spilled volume is usually more than 1,000 cubic meters.

There have been many large and small oil spills since the expansion of crude oil production in the mid-1800's. The amount of oil spilled in a large oil spill incident may be up to 500,000 to 1,000,000 cubic meters. (Table 2.1) In global rankings based on oil volume, the Rayong oil spill is ranked 158th.[8]

Table 2.1: List of 10 Largest Oil Spills by Volume (not related to war) and the Rayong Oil Spill

Rank	Spill/vessel	Location	Date	Amount Spilled (Cubic meters)	Refs
1	Lakeview Gusher	United States	14 March 1910 – 10 September 1911	1,426,800	[9]
2	Deepwater Horizon	United States	20 April 2010 – 15 July 2010	847,000-1,035,000	[10]
3	Ixtoc I	Mexico	3 June 1979 – 23 March 1980	529,950	[11]
4	Atlantic Empress / Aegean Captain	Trinidad and Tobago	19 Jul 1979	332,920	[2, 12]
5	Fergana Valley	Ozbekistan	2 March 1992	320,000	[13]
6	ABT Summer	Angola	28 May 1991	300,000	[2, 12]
7	Nowruz Field Platform	Iran	4 February 1983	300,000	[2, 12]
8	Castillo de Bellver	South Africa	6 August 1983	292,000	[12]
9	Amoco Cadiz	France	16 March 1978	263,000	[12]
10	MT Haven	Italy	11 April 1991	167,000	[2, 12]
158	Rayong Oil Spill	Thailand	27 July 2013	46-189	[1, 8]

2. Oil Spill Effects

A crude oil spill can directly affect the environment and human health, exposing individuals to toxicants from crude oil and oil dispersants used for oil spill cleanup.[8, 14] Oil spills can also indirectly affect the socio-economic fabric of the contaminated area.[1, 14-16] For example, a spill might result in temporary or permanent damage to fishing or marine recreation industries, causing unemployment and regional economic losses.[15, 17] Examples of impacts of oil spills on the environment and regional and national economy are described below.

Environmental Impacts

Oil spills can affect a variety of marine organisms, including plants, coral reefs, plankton, fish, turtles and birds.[18, 19] The toxicokinetic and toxicodynamic processes in marine species are varied, and thus result in differences in oil impacts on different species. For example, many coral communities near the Deepwater Horizon oil spill showed signs of stress, including tissue loss, sclerite enlargement, bleached and excess mucous production, acutely[20] and 2 years[21] after the oil spill. Whereas, many types of phytoplankton experienced an increase in mortality and growth inhibition after exposure to crude oil contaminated water in laboratory experiments.[4] Mammals are also affected as reported in a study following the Deepwater Horizon oil spill where about 1,000 cetaceans (marine mammals) including dolphins were stranded or dead immediately after the incident.[22]

A study from the Exxon Valdez oil spill showed an increase in mortality of sea otters 10 years after the incident.[23] Increased PAH concentrations associated with unusual mortality events (UME) were found in fish and their embryos after the Heibei Spirit (Korea),[24] Prestige,[25] Exxon Valdez,[26] and Deepwater Horizon oil spills.[22] Oil-related lethality of up to 100 sea turtles was reported after the Deepwater Horizon oil spill.[27] In waterfowl, the cause of death can often be the loss of thermal insulation due to oil-coated feathers.[28, 29] After the Deepwater Horizon oil spill, more than 4,000 seabirds were contaminated with oil and about 2,000 of those were found dead.[27]

Laboratory studies suggested that a reason for UME in marine animals might be the genotoxic and immunotoxic effects from PAHs found in crude oil.[24, 25, 30, 31]

Economic Impacts

The major direct economic cost of an oil spill is usually in the form of damage to natural resources and local industries – often seafood or tourism – and subsequent compensation costs by the responsible oil company. After the Deepwater Horizon oil spill, the BP Exploration & Production company was charged an unprecedented $5.5 billion USD Clean Water Act penalty and up to $8.8 billion USD in natural resource damages.[32] Meanwhile, the estimated short-term economic impact of the Deepwater Horizon oil spill on the local seafood industry was $952 million USD lost due to decreasing sales, $21 million USD lost due to decreasing income, and 9,315 jobs lost due to the temporary stop of seafood production.[33] In addition, the indirect loss from recreational fishing was about $585 million USD.[17] In the Prestige oil spill off the coast of Spain, the Galicia government paid a total of €146 million Euros for fishing activity compensation and oil spill cleanup.[15] The fishing income lost from the Prestige oil spill was estimated to be around €81 million Euros.[15]

3. Human Health Impacts

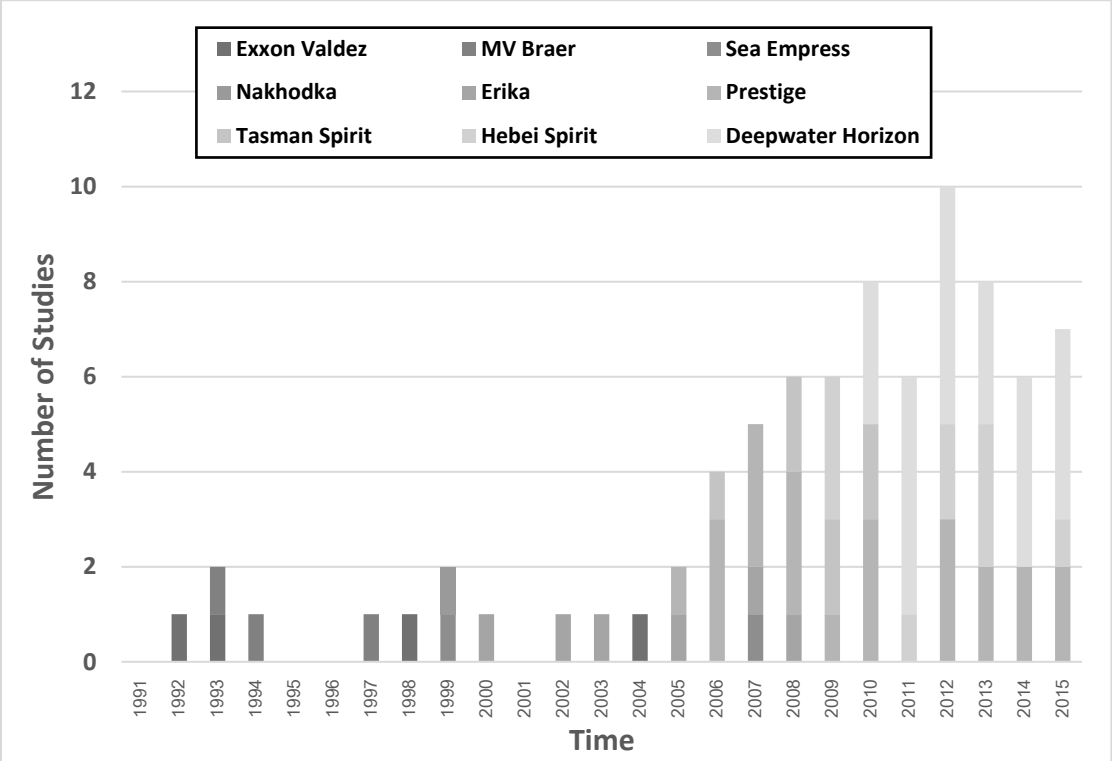
This dissertation focuses on human exposure and health consequences from an oil spill off the coast of Thailand. Therefore, the human health impacts will be discussed in more detail than the environmental and economic impacts. Several oil spills specifically related to human health effects will be discussed referring to the incidents listed in Table 2.2 below:

Table 2.2: Oil Spill Incidents with Research Related to Human Health (by amount of spill) (modified from Aguilera et al, 2010 and Laffon et al, 2016)[34, 35]

Incident	Date	Country	Amount spilled (Cubic meters)
Deepwater Horizon	20 April 2010	USA	847,000-1,035,000
MV Braer	5 January 1993	UK	100,000
Sea Empress	15 February 1996	UK	85,000
Prestige	19 November 2002	Spain	74,000
Exxon Valdez	24 March 1989	USA	44,000
Tasman Spirit	26 July 2003	Pakistan	44,000
Erika	12 December 1999	France	24,000
Hebei Spirit	7 December 2007	Korea	13,000
Nakhodka	2 January 1997	Japan	7,000

There have been about 11,000 research reports in the oil spill literature since 1967.[36] The number of oil spill-related health impact studies increased steadily from 1974 to 2014 at a rate of about 5% per year.[36] One-third of these studies focused primarily on biological endpoints, while only 1% of studies was related to human health.[36] However, recently due to the Deepwater Horizon oil spill, the number of studies related to human health has increased (Figure 2.1).

Figure 2.1: Trend in Number of Oil Spill Studies related to Human Health (Adapted from Murphy D. et al. 2016)[36]



Even with the increasing number of human health studies, information on long-term health effects is still lacking.[36] One of the few studies from the Prestige oil spill investigated long-term health outcomes such as chromosomal damage[37, 38] and persistent respiratory symptoms[39] at 4 to 7 years after the oil spill.

Acute Symptoms

Many studies from oil spill incidents have used structured questionnaire surveys to examine acute symptoms in cleanup workers and residents exposed to oil spills.[40, 41] The prevalence of symptoms related to, or increased in, oil-exposed subjects by the order of decreasing frequency are summarized as follows: [40, 41]

- Respiratory symptoms, including throat irritation, sore throat, other throat symptoms, cough, shortness of breath and runny nose
- Eye symptoms, including eye irritation and sore, itchy or reddened eye
- Headache

- Skin symptoms, including irritation, itching, and rash and skin lesions
- Nausea
- Dizziness
- Tiredness or fatigue
- Muscle pain and injuries, including back pain

The prevalence of these acute symptoms is sometimes associated with days of cleanup work and duration of work.[40, 41] Females and children may be susceptible populations with a tendency to have higher prevalence of symptoms.[40] A study from the Heibei Spirit oil spill found that urinary VOC and PAH metabolites were associated with some of these symptoms.[41]

Short and Long-Term Respiratory Symptoms

Spirometry is the primary method used for measuring pulmonary function and respiratory outcomes in clinical and research settings.[42] The parameters of spirometry used for measuring pulmonary function are peak expiratory flow (PEF), forced expiratory volume in the first second (FEV1), forced vital capacity (FVC) and forced expiratory flow (FEF) $_{25\text{-}75\%}$.[42, 43] The $FEF_{25\text{-}75\%}$ is the forced expiratory flow during the middle half of the FVC.[42] The ratio FEV1/FVC% is the ratio of the forced expiratory volume in one second to the forced vital capacity, recorded as a percentage.[42]

Two studies from the Braer oil spill in 1993 did follow-up respiratory health exams, measuring PEF at 3-56 days and 6 months after the oil spill in children[44] and residents[43] residing near the shipwreck. PEF in exposed populations from the two studies were within the normal range.[43, 44] Meo et al. studied lung function in 31 exposed subjects a year after the Tasman Spirit oil spill comparing them with age, height and weight matched controls.[45] The study found a decline in FEV1, FVC and $FEF_{25\text{-}75\%}$ in the exposed subjects, compared to the controls.[45] A study conducted 1.5 years after the Hebei Spirit oil spill (Jung et al) reported significantly lower FEV1 and higher prevalence of asthma attacks in children who lived near the oil spill than among children who lived farther away from the oil spill site.[46] Two studies from the Prestige oil spill (Zock J.P.

et al, 2012, 2014) examined the persistent respiratory symptoms and pulmonary function of clean-up workers 5 to 6 years after the oil spill.[39, 47] Both studies showed no significant differences in respiratory symptoms and pulmonary function between the clean-up workers and controls.

Psychological and Mental Health

Many studies from various oil spill incidents have explored the psychological/mental impacts of oil spills.[40, 41] Common mental conditions examined in studies include depression, anxiety disorders, event-related psychological stress, and post-traumatic stress disorder (PTSD).[40, 41] Depression was generally assessed by the Center for Epidemiologic Studies Depression (CES-D) score[48] and the patient health questionnaire (PHQ)-9.[49] Anxiety disorders were examined by State-Trait Anxiety Inventory (STAI-X-1) or generalized anxiety disorder (GAD)-9.[41] PTSD was usually evaluated using the Posttraumatic Diagnostic Scale (PDS). General mental health was evaluated with Symptom Checklists (SCL-36 and SCL-90-R), the Short Form-36 (SF-36) and/or the Psychological Well-Being Index (PWI).[40, 41] Generally, studies from oil spill incidents found that the prevalence and symptom score for depression, anxiety disorders, psychological stress and PTSD were higher in the oil-exposed population than in unexposed controls.[40, 41] In addition, adequate social and economic support helped the exposed population and communities to better cope with stress from oil spills.[41]

4. Biomarkers of Crude Oil Exposure and Effect in Blood and Urine

Metabolites of PAHs, VOCs and heavy metals have been measured in several studies from the Hebei Spirit oil spill. [40, 41] Few studies from other oil spill incidents measured these metabolites. Ha et al. reported that in cleanup volunteers at the Hebei Spirit oil spill, the levels of t,t-muconic acid, (a metabolite of benzene), mandelic acid (a metabolite of VOCs), and 1-hydroxypyrene (a metabolite of PAHs) were higher in the urine collected after the cleanup than in the urine collected before the cleanup.[50] Lee et al. conducted a cross-sectional study to compare the levels of VOC and PAH metabolites in urine from residents and volunteers who wore or who did not wear personal protective equipment (PPE). The study found that the concentration of VOC and PAH metabolites

in urine was not different between the residents who wore or did not wear PPEs.[51] However, the study was limited due to the fact that it was conducted in the later weeks of oil spill cleanup when the levels of PAHs and VOCs were expected to be low. Similarly, other studies from the Hebei oil spill examined internal dose biomarkers of PAHs and VOCs at least 2 weeks after the cleanup.[41] The half-life of VOCs and volatile PAHs are relatively short, ranging from 6 to 24 hours,[52] Therefore, the low levels of biomarkers in those studies were as expected. From the Deepwater Horizon oil spill, D'Andrea and Reddy reported that 80% of 117 cleanup workers had detectable levels of urinary phenol, a metabolite of benzene which is normally found only in workers exposed to benzene at more than 1 ppm.[53]

A number of biomarkers of biological effect have been examined in various studies related to oil spills, including complete blood cell counts (CBC), hemoglobin concentration, hematocrit, liver function tests, and renal function tests.[34, 35, 54] The urinary biomarkers of oxidative stress that were assessed in a few studies were malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG). From the Tasman Spirit oil spill, Khurshid et al. reported that lymphocyte and eosinophil counts in people living near the oil-contaminated beaches were slightly increased, compared to the standard value.[55] A study of Noh et al. from the Hebei Spirit oil spill reported that levels of MDA and 8-OHdG were positively associated with the levels of 1-OHP.[56] D'Andrea and Reddy reported in their two studies from the Deepwater Horizon oil spill that platelet counts were decreased, while hemoglobin and hematocrit levels were increased, in the oil spill cleanup workers.[53, 57] In addition, the liver enzymes AST and ALT and creatinine were above the upper limit of normal in 15%, 31% and 23% of the workers, respectively.[53, 57]

Several biomarkers of genotoxicity have been assessed in oil spill volunteers and workers, including the comet assay, the micronucleus (MN) test, and sister chromatid exchanges (SCE).[34, 35] In workers from the Braer oil spill, there was no evidence of DNA damage reported in the only paper available, however, the sample size was small (n=26).[58] In the Prestige oil spill, an early study from years 2006-2008 showed evidence of higher levels of DNA damage (by the comet assay

and the MN test) and altered frequencies of DNA repair genetic polymorphisms in volunteers and hired workers compared to controls.[34, 59, 60] In addition, the genotoxicity results were influenced by sex, age and cigarette smoke.[40] A follow-up study in year 2010 from the Prestige oil spill by Rodriguez-Trigo et al, found structural chromosomal alterations in the circulating lymphocyte of local fishermen.[61] However, two further studies at 6 and 7 years after the spill (in year 2014 and 2015) did not find any chromosomal aberrations in these fishermen.[37, 38] Thus, these findings might suggest that DNA damage in bone marrow cells due to crude oil was transient and reversible. Conversely, these findings may indicate that the initial findings were not correct.

Biomarkers of endocrine effects, including cortisol and prolactin, were used as evidence of endocrine disruption in various studies of the Prestige oil spill. No studies from other oil spill incidents examined endocrine effects.[34, 35] The Prestige results indicated that serum cortisol and prolactin levels decreased in cleanup workers compared to unexposed controls.[43,44,62]

The genotoxicity studies mentioned above were mainly post-spill cross-sectional studies without follow up or baseline data. In addition, no quantitative exposure assessment was performed for the cases and control group. Therefore, it is difficult to conclude that the observed genotoxic effects were due to exposure to toxicants from the oil spill cleanup.

5. General Cleanup Procedures

Several methods are available and can be used in combination to contain, restrict and eliminate crude oil that has contaminated environmental media, including soil, rock and water.[1, 7, 63, 64] Containment and dispersal are the primary methods used immediately after most oil spill incidents. Containment involves the use of booms wrapped with hydrophobic material, including foam and plastic. So-called “hard booms” are used for the sole purpose of oil containment (Figure 2.2). Whereas, sorbent booms are made of material which can absorb oils and float on water such as polypropylene. Fire booms, made from fire resistant material, are used to contain crude oil before burning.

Oil dispersants are emulsifiers, such as butoxyethanol or kerosene, that can link hydrocarbon (uncharged) bonds to the covalent (charged) bonds of water.[8] Examples of commercial oil dispersants are Superdispersant-25[64] and COREXIT E-series[63] composed mainly of 2-butoxyethanol (10-30%) and dioctyl sulphosuccinate (10% as a surfactant).[8, 63] During oil spill cleanup, the dispersants are distributed by aerial or water-level spraying. Important factors to consider before spraying are distance from coral reefs, seawater wave power, and depth of the water.[7] The amount of dispersant used must be properly estimated and monitored since excess exposure, such as occurred in the Deepwater Horizon oil spill cleanup, might induce stress and UME in fishes,[65, 66] corals[67] and their larvae.[67] COREXIT 9500 and 9527 were used in the Deepwater Horizon oil spill,[32] whereas, Superdispersant-25 and Slickgone NS were used in the Rayong oil spill.[1, 64]

Figure 2.2: Containment Boom[43]

Removal or elimination methods involve several procedures, including skimming, controlled burning, vacuuming, shoveling, high-pressure hot water spraying, absorbing and bioremediation, to eliminate or accelerate the degradation of crude oil and its components.

` Oil skimmers are devices that are attached to tow boats and used to separate oil that floats on the surface of water from water. Skimmers can be divided into 2 major types, pumping and adsorbing skimmers. Pumping skimmers are used on very thick oil films (Figure 2.3) and vacuum oil off the surface of the water, whereas, adsorbing skimmers are used on thinner oil films, and are made of adsorbing hydrophoic materials such as Teflon (Figure 2.4).

Figure 2.3: Pumping Skimmer[43]

Figure 2.4: Adsorbing Skimmer[8]

Controlled burning, or in situ burning, was the method used in the Deepwater Horizon oil spill. This involves burning the contained oil at the site of the spill.[68] However, this method has the potential for widespread air pollution in the form of particulate matter or VOCs.[32] In addition, the combustion byproducts, carbon dioxide and carbon monoxide, can contribute to climate change.[8] Therefore, the appropriateness of the method for widespread use is still questionable.

Manual removal, vacuuming and on-site treatments are approaches that require the use of vacuum trucks or other vacuum equipment, and hand tools to remove crude oil-contaminated water or soil (Figure 2.5). Contaminated water can be treated initially with portable on-site wastewater treatment systems, and subsequently transferred to standard wastewater treatment plants for further treatment.[8] Absorbing spilled oil requires the use of absorbing materials that can absorb oil and separate it from water. A common example is the use of absorbent pads made from polypropylene to absorb crude oil on beaches.

Figure 2.5 Oil Spill Cleanup by Manual Removal (From Thai Naval Medicine Department)

High-pressure hot water treatment of oil-contaminated beaches and rock is used to distill the oil and drain it into an area with installed booms. However, this method is not ideal because it reintroduces toxicants from crude oil into the ambient air and water.

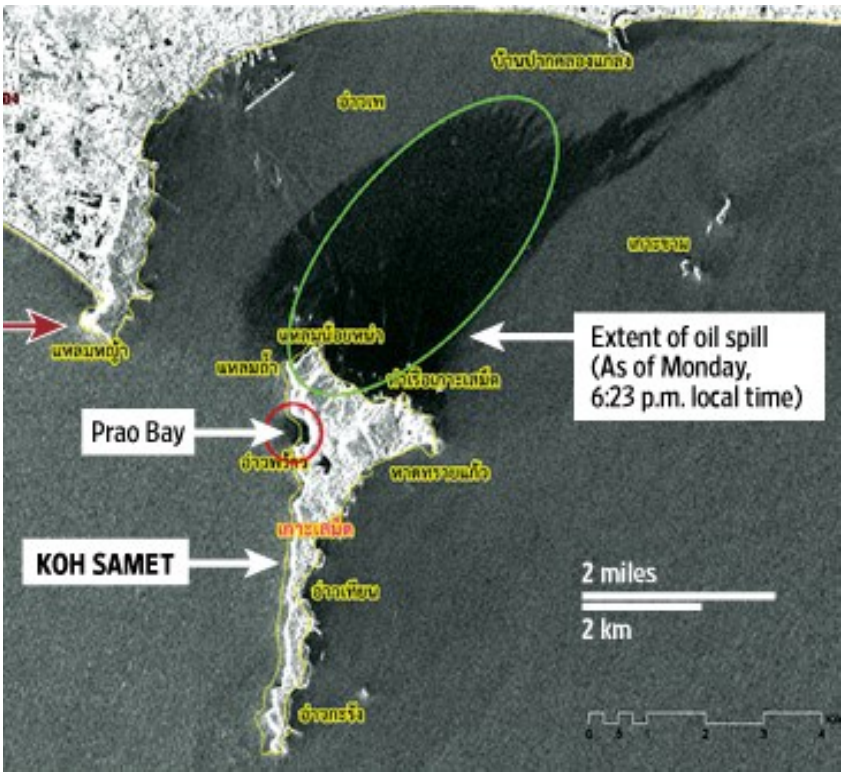
Bioremediation is a method that adds natural nutrition or substances that can accelerate the oxidative reactions or the decay processes of crude oil components. Common examples of bioremediation agents are hydrocarbon-degrading bacteria, micronutrients, and biodiesels.[69] The usefulness of these bioaugmentation and biostimulation substances is somewhat controversial. In some studies or situations, these substances have not proved to be helpful for the decay and degradation of crude oil, especially in dealing with thick-layered crude oil.[70, 71]

B. Rayong Oil Spill Background

1. Causes and Oil Spread

On 27 July 2013, the pipeline connecting an offshore oil platform to a tanker, operated by PTT Global Chemical (PTTGC), a corporation owned by the government of Thailand, leaked and caused crude oil to spill into the sea of Rayong off the coast of Thailand. The leakage was found at a single point mooring that discharged the crude oil from the vessel to the refinery.[64] The crude oil covered an area of approximately 20 square kilometers (Figure 2.6) and washed ashore on the island of Samet in an area called "Ao Prao" on 28 July 2013.[1] Although PTTGC estimated that the amount of oil spilled was about 50 cubic meters or 336 barrels[64], other experts believe the true volume to be as high as 190 cubic meters or 1,200 barrels.[1] The on-water containment procedures started on 27 July 2013, the same day as the oil spill. However, the containment efforts failed and the oil slick washed ashore on the morning of 29 July 2013 (Figure 2.6) when the on-land cleanup began[1] and continued until 26 August 2013.[1]

Figure 2.6: Satellite Photograph of Oil Spill Off the Coast of Rayong Province, Thailand, After Contaminating Beaches at Ao Prao Bay (29 July, 2013)



2. Cleanup Procedures

To mitigate the situation, the pipeline valves were closed as soon as the leakage was detected.[64] Then oil spill cleanup procedures were followed which included on-water and on-land cleanup. The on-water cleanup initiated by the PTTGC oil company and the Thai National Emergency Response Team used containing booms, skimmers and oil dispersants.[64] The oil dispersants used in the cleanup were 6,930 liters of Superdispersant-25, that contains the potential toxicants 2-butoxyethanol and dioctyl sulphosuccinate, and 30,612 liters of Slickgone-NS, that contains the potential toxicants kerosene and sodium dioctyl sulphosuccinate.[72] The cleanup schedule was as follows:[64]

27th July 2013: skimmers were used to collect oil from the sea surface. Oil dispersants were sprayed by ships and aircraft.

28th July 2013: floating booms were placed to contain the spill. Ships from the PTTGC company, the Royal Thai Navy, the Marine Department and International Regulations for Preventing Collisions at Sea (IRPC) and aircraft from Oil Spill Response Limited (an international industry-funded cooperative that deals with oil spills) from Singapore coordinated to contain and clean the oil slick. However, the containment was not completely successful and the crude oil washed ashore at the Prao bay area on Samet Island.

29th July 2013: the on-land cleanup was implemented by the Thai Navy and Thai government. Procedures included shoveling contaminated rocks and soil, vacuuming oil, high-pressure water cleaning and using absorbent pads to collect and remove crude oil. The workers who cleaned the contaminated area included company employees, soldiers from the Royal Thai Navy, and citizen volunteers.[1, 73]

29th July - 2nd August 2013: (Day 1 to Day 5 of on-land cleanup) Intensive cleanup by shoveling, vacuuming, high-pressure water and absorbent pad use.

Figure 2.7: On-Land Cleanup of Rayong Oil Spill, 30 July 2013 (From Thai Naval Medicine)

3rd – 5th August 2013: (Day 6 to Day 8 of on-land cleanup) The oil debris collected was moved by cranes and bulldozers to Higgins boats (landing craft) and the frigate HTMS Angthong 791, which transferred the oil contaminated debris from Samet island to PPTGC refineries on the mainland for further treatment (Figure 2.8).

Figure 2.8: Transferring of Oil Contaminated Debris by Landing Craft and HTMS Angthong (From Thai Naval Medicine)


6th – 26th August 2013: (Day 9 to Day 29 of on-land cleanup) continued collection of contaminated debris.

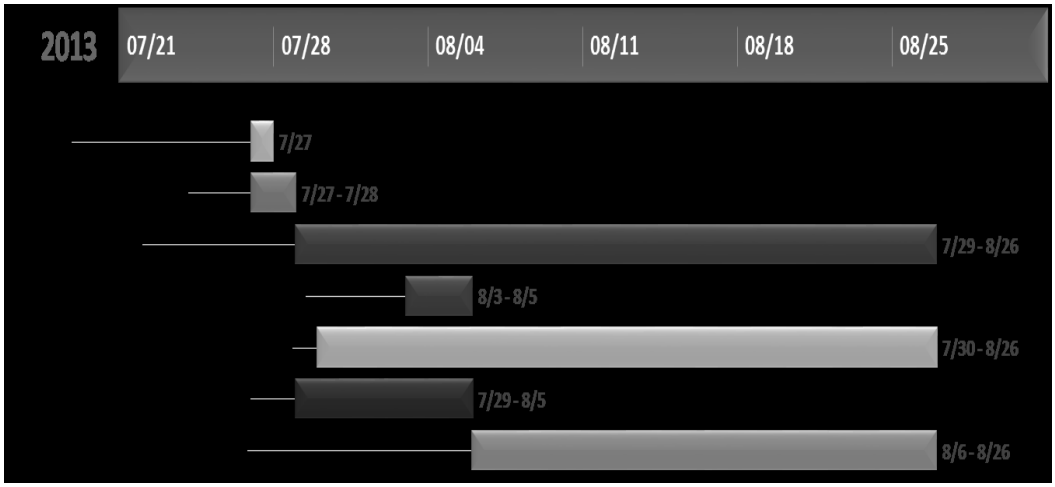
27th August 2013: The on-land cleanup ended. Subsequent environmental monitoring and sampling of water and air were performed by the Thai Pollution Control Department of the Thai government. Seafood sampling was performed by the Thai Medical Science Department of the Thai government. However, detailed data was not reported to the public.[1]

3. Previous Health Follow-up and Surveillance Plan

The Rayong Provincial Public Health Office, Rayong Hospital, the Thai Bureau of Occupational and Environmental Diseases, and the Thai Naval Medical Department designed a health surveillance plan for the oil-spill cleanup workers. They collected urine samples post-shift to measure urinary tran,tran-muconic acid (t,t-MA), an internal dose biomarker of benzene, and administered a questionnaire survey post-shift asking about demographic factors, cleanup jobs, days worked, hours worked, personal protective equipment (PPE) use, underlying diseases, smoking status and acute symptoms. However, the health surveillance protocols were approved a day after the on-land cleanup began. The earliest day with available urine and questionnaire data was 30 July 2013 – the day after cleanup began. Blood sampling, chest radiography, and more detailed questionnaires started on 5 August 2013 -- 7 days after the cleanup began. Detailed

information on the workers and questionnaires will be discussed in chapter 3. The timeline of the spill, the cleanup and the health surveillance plan is summarized in the calendar (Figure 2.9):

Figure 2.9: Rayong Oil Spill Cleanup Sequence of Events (27 July 2013 – 26 August 2013)



Note: On-water containment included oil dispersants, foam injection and containment booms.

Note: Contaminated sand and water were transferred to a refinery on the mainland for further treatment.

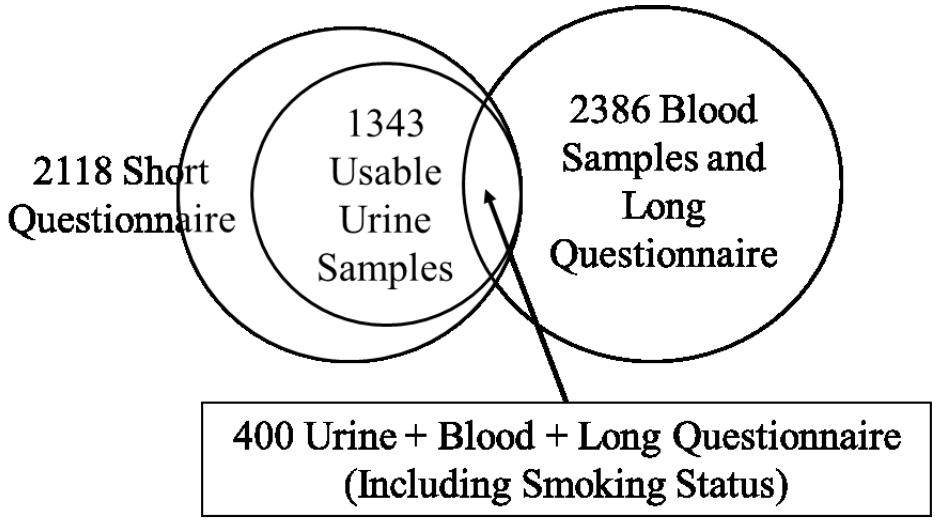
Some of the cleanup workers were asked to voluntarily provide a urine sample immediately post-shift. The samples were tested for urinary t,t-MA in Thai government laboratories. In addition, a personal medical history and screening physical examination by organ system for every worker was performed by Thai government physicians. Moreover, workers were asked to voluntarily provide their blood for further laboratory tests, including complete blood counts (CBC), liver enzymes (AST and ALT), blood urea nitrogen (BUN) and creatinine. Furthermore, a chest x-ray was performed in workers who agreed to it.

For long-term follow up, the workers who worked in the first week of cleanup (29th July-5th August) were advised to have their blood tested for CBC every 3 months in the first year after the oil spill and once every year after that. Their liver enzymes, BUN, creatinine and chest radiography were to be performed every year for at least 5 years after the Rayong oil spill. The

rest of the workers were advised to have their blood tested for CBC, liver enzymes, BUN, and creatinine measured and chest radiography at the 2nd and 5th years after the oil spill.

Data from this health surveillance program was available for our research, including questionnaires, urinary t,t-MA and (partial) creatinine measurements. There were a total of 2,118 records from a short post-shift questionnaire (including demographic factors, days of cleanup worked, job descriptions and acute symptoms) collected at the same time as the urine sample collection. Of the 2,118 records, only 1,343 urine samples were available for further laboratory analysis as shown in Figure 2.10. There was another subset of workers containing 2,386 records which included blood samples and a long questionnaire (including smoking status) at the end of cleanup in September, 2013. This subset did not include urine samples except for a small number (n=400) of workers.

Figure 2.10: Venn Diagram Summarizing Worker Population with Urine Samples and Blood Samples



The demographic factors of the 2,118 records are shown in Table 2.3. Of the 2,118 worker records, 88% of them were male and the median age was 28 years old. Most of them were military personnel or PTTGC employees, and worked during day 2 to 4 of cleanup. The distribution of these 2,118 worker records by age, sex, background and days of cleanup were similar to the distributions among the 1,343 urine samples, as described in Chapter 3.

Table 2.3: Demographic Factors of Cleanup Workers from Short Questionnaire

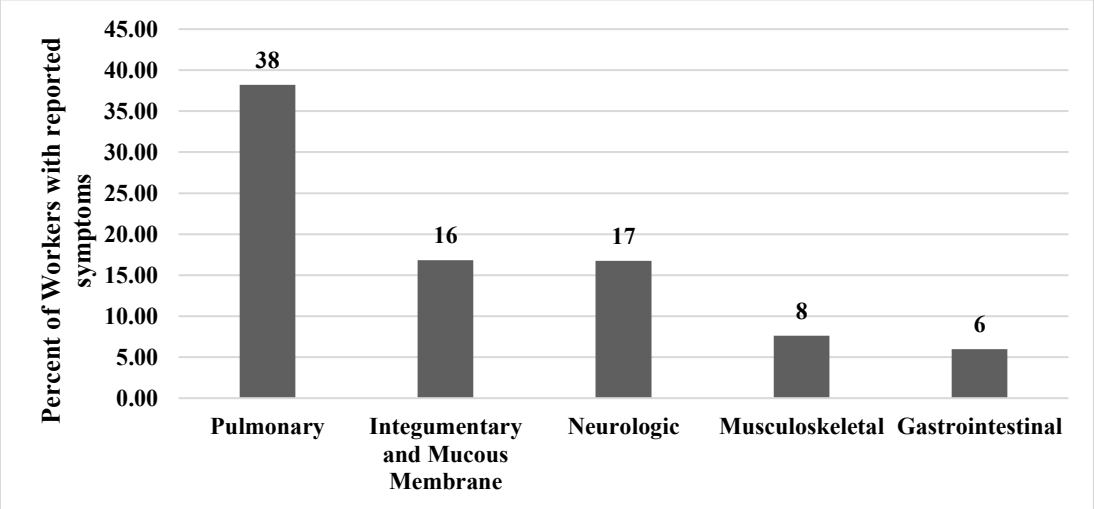
Demographic Factors	Descriptions	Number of Workers	Percent
Total		2118	100.0
Age	Median (1st-3rd Quartiles)	28.0 (22.0-40.0)	
	Unknown Age	44	2.1
Sex	Male	1869	88.2
	Female	202	9.5
	Missing	47	2.2
Background	Military Personnel	968	45.7
	PTTGC Company Employees	782	36.9
	Citizen Volunteers	359	16.9
	Unknown	9	0.4
Days of Cleanup	Day 2-4	1159	54.7
	Day 5-7	446	21.0
	Day 8-14	300	14.2
	Day 15-21	117	5.5
	Day 21-28	85	4.0
	Missing	11	0.5

4. Previous Studies from the Rayong Oil Spill

Only two studies have been published so far regarding the Rayong oil spill cleanup workers, using data from the questionnaires and the health examinations performed as part of the surveillance program. A peer-reviewed study by Sithisarankul et al (2014), published in English, is a preliminary report of workers characteristics, urinary t,t-MA levels, and their self-reported acute symptoms, using a dataset of 2,118 workers who voluntarily provided their urine samples.[74] Almost all of them (90.2%) were male with occupation reported as a military professional (45.7%).[74] The mean age of the workers was 31.9 years and 82.2% of the workers worked on day 1 to day 5 of the cleanup (29 July to 2 August).[74] The urinary t,t-MA levels were reported in categories using the cut-off at 500 ug/gCr of urinary t,t-MA and the limit of detection was unclear. Only 1 worker was reported to have a t,t-MA level more than 500 ug/gCr. Detailed values of

individual worker's t,t-MA concentration were not reported in the study. The acute symptoms were grouped and reported by organ system. The organ system with the most frequently reported symptoms was the pulmonary system (38.2%). The organ system with the least frequently reported symptoms was the gastrointestinal system (6.0%). Figure 2.11 summarizes the acute symptoms by organ systems in the order of numbers of cases from Sithisarankul's study.

Figure 2.11: Percentage of Workers with Reported Symptoms by Organ Systems. (Adjusted from Sithisarankul, 2015)[74] (n=2,118)



Being a preliminary report, the study of Sithisarakul et al is somewhat limited, showing only summary data and descriptive statistics. However, the study reports an interestingly high prevalence of pulmonary symptoms which should be further explored. Further study designs with inferential statistics are needed to assess associations between the acute symptoms and estimated exposure days and PPE use.

A second study of Rayong oil-spill workers conducted by Rheanpumikankit et al, was published in a Thai regional medical university journal. The study examined an expanded group of workers from those studied by Sithisarakul et al that included 2,409 workers who voluntarily consented to have their blood tested. The study classified cleanup workers into 3 separate groups by days of cleanup work, hours worked, and job descriptions. The high exposure group included workers whose job descriptions included vacuuming, shoveling or skimming of oil, and who

participated for at least 6 hours/day for at least one day during Days 1-5, and the oil transporters who worked during Days 6-8. The medium exposure group were workers whose job descriptions included sand removal, journalist, or supervisors who participated for at least 6 hours/day for at least one day during Days 1-5. Whereas, the low exposure group included workers who participated for less than 6 hours during Days 1-5 and the workers who worked during Days 6-29 regardless of duration.

The focus of this study was the initial and follow-up results of CBC, creatinine and liver enzymes. The acute symptoms were also vaguely reported as the number of cases and percentages.[75] However, some of the results seem doubtful since irritating nose symptoms were reported as 100% prevalent in all 2409 workers. The laboratory results were compared among the three pre-defined exposure groups mentioned above. The study reported that, immediately after the Rayong oil spill, the proportion of workers with serum AST and ALT levels more than 35 IU/liters was higher in the high exposure group than in the medium and low exposure groups. The proportion of workers with anemia and serum creatinine more than 1.5 mg/dL was not different among the 3 groups. Only the high exposure group was retested at one year of followup for CBC, serum creatinine, AST and ALT. However, there was no change in proportion of workers with AST and ALT levels more than 35 IU/L as compared to the baseline post oil spill results. This suggests that the observed larger proportion of workers with high AST and ALT in the high exposure group at baseline could be due to unrelated factors rather than exposure to toxicants from the Rayong oil spill. Other potential factors that can cause an increase in AST and ALT levels are alcohol consumption and liver diseases (e.g., non-alcoholic steatolic hepatitis (NASH)), caused by obesity and hyperlipidemia. Furthermore, the fact that the medium and low exposure groups were not followed up at one year after the oil spill, means that there was no control to compare with the high exposure group.

The paper by Rheanpumikankit et al. had a number of other limitations. Firstly, the classification of exposure groups was not clear or practical. The study differentiates high and

medium groups only by job descriptions. Misclassification might be an issue because participants might perform several jobs in the same day. Another issue is the classification by the working hours of the workers. As mentioned above, to satisfy the criteria for high and medium exposure the cleanup workers had to work at least 6 hours. About 20% of workers did not provide information on the duration of cleanup jobs, making it hard to classify by the proposed exposure group criteria. Secondly, the blood samples were not drawn on the same day as cleanup work. Some of the samples were taken up to 30 days after the day worked. This point was not mentioned and accounted for in Rheanpumikankit's paper. Thirdly, anemia, creatinine, AST and ALT were used as categorical variables, thus reducing the statistical power of these measures as compared to using continuous variables. In addition, the classification of anemia, high creatinine, high AST and high ALT were not clearly described and did not use standard classifications. Furthermore, WBC and platelets are more sensitive to the exposure to benzene than is hemoglobin.[76] Therefore, the study should examine the number of WBC and platelets, in addition to hemoglobin concentration.

The proposed health surveillance plan from the Rayong provincial hospital, the Thai Naval Medical department and the Rayong local health office could be improved. For long-term follow up, all workers should be advised and given the same blood exams every year. Currently, there is no evidence that the workers who worked in the first week of cleanup are more likely to have abnormal CBC and liver enzyme levels than the workers who worked during the subsequent weeks. In terms of risk assessment, all workers should be considered exposed to toxicant levels sufficient to cause adverse health effects until there are enough data to disprove potential adverse health effects.

C. Crude Oil Composition, Potential Toxicants and Confounding Factors

Crude oil is composed of 84-87% carbon, 11-14% hydrogen, 0.06-2% sulfur, 0.1-2% nitrogen, 0.1-2% oxygen and trace amounts of metals.[77] It is a complex mixture of hydrocarbons, some of which may affect human health, including VOCs such as benzene, and PAHs such as pyrene and benzo[a]pyrene.[78] The composition of crude oil is summarized in Table 2.4.

Table 2.4: Crude Oil Composition (Adapted from JH Kim et al. 2012)[78]

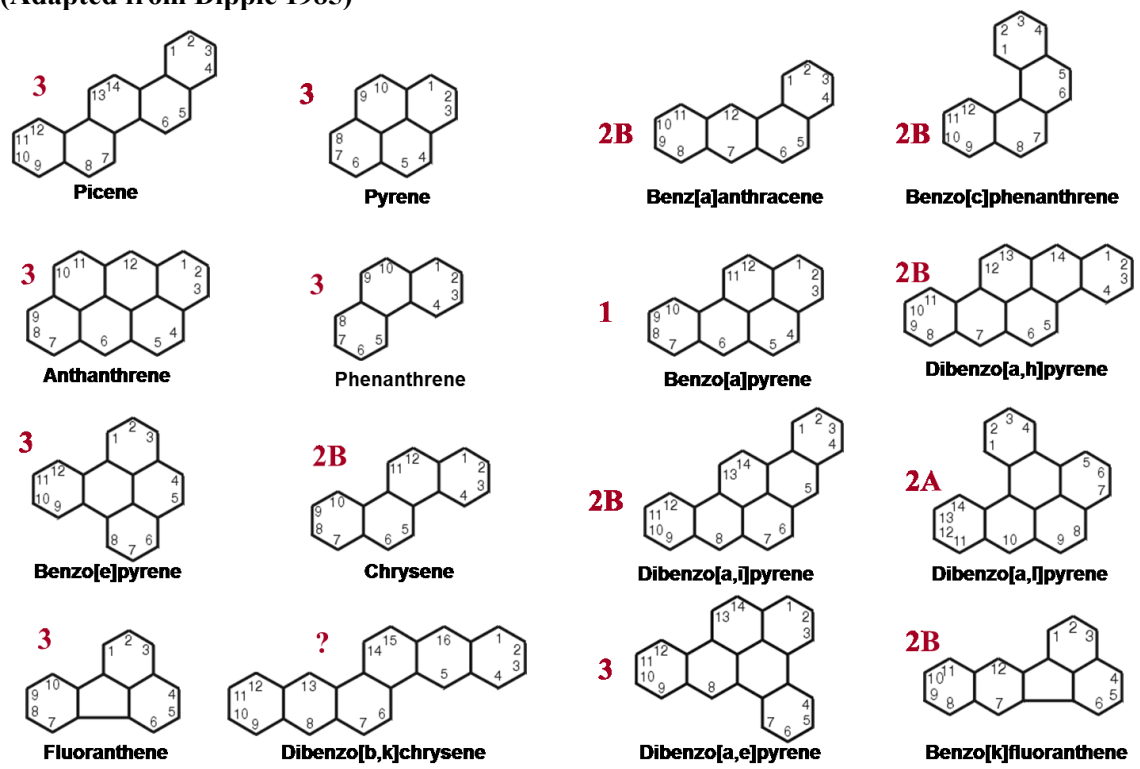
No.	Material	Weight Fraction	Molecular weight (g/moL)	Average density (kg/m3)	Average solubility (g/m3)
1	Paraffin (C6 - C12)	0.15	86-170	710	4.755
2	Paraffin (C13 - C25)	0.15	184-352	770	0.007
3	Cycloparaffin (C6 - C12)	0.2	84-164	810	28
4	Cycloparaffin (C13 -C23)	0.2	156-318	900	0.5
5	Aromatic mono and dicyclic (C6 - C11)	0.042	78-143	940	890
6	Aromatic polycyclic (C12 - C18)	0.03	128-234	1000	6.25
7	Naphtheno - aromatic (C9 - C25)	0.07	116-300	980	0.5
8	Residual including heterocyclics	0.15	300-900	1010	0
9	Benzene	0.001	78.11	878.6	800
10	Toluene	0.003	92.14	867	526
11	Ethylbenzene	0.001	106.17	867	206
12	Ortho-,meta-,para-Xylene	0.003	106.17	870	0

Of these components, we selected two toxicants, benzene and aromatic polycyclics, that can affect human health and have internal dose biomarkers measurable in urine. In addition, the International Agency for Research on Cancer (IARC) has classified benzene and benzo[a]pyrene (a PAH) as group 1 carcinogens[79], known to cause cancer in humans. A few studies from previous oil spills have measured internal biomarkers in urine of PAHs and benzene. Two of the studies from the Hebei oil spill (Ha et al. and Lee et al.) measured the levels of t,t-MA and 1-hydroxypyrene in the urine of cleanup volunteers.[50, 51] A study from the Deepwater Horizon oil spill by D'Andrea and Reddy measured urinary phenol, a metabolite of benzene.[53] Since my dissertation focuses on PAHs and benzene, the two toxicants will be discussed in detail.

1. Polycyclic Aromatic Hydrocarbon (PAH)

Polycyclic aromatic hydrocarbons, or PAHs, are a group of over 100 different organic compounds, consisting of two or more fused benzene rings, arranged in various forms.[80, 81] In general, the sources of PAHs are incomplete combustions of organic materials, formed by various processes, including power production, coke production, iron/steel foundries, forest fires, internal combustion engines, cooking processes, food production (smoked products) and tobacco smoke. [82, 83] Physical and chemical properties of PAHs vary based on the numbers and arrangement of aromatic rings, molecular weights and functional groups attached to the benzene rings.[84] IARC has classified several PAHs as known (group 1), probable (group 2A), possible (group 2B), or unknown (group 3) human carcinogens as indicated in Figure 2.12.[80] Although animal studies are conducted to test the carcinogenicity of individual PAHs[85], humans are exposed to mixtures of PAHs rather than individual PAHs. Thus, epidemiological studies are conducted to link cancer outcomes to PAH mixture scenarios such as lung cancer mortality to coke oven emission[86, 87] or cigarette smoke.[88] General populations are exposed to PAHs via cigarette smoke, indoor air pollution from heating and cooking, ingestion of broiled or smoked foods and contaminated drinking water.[89]

Figure 2.12: Structures and Nomenclature of 16 PAHs and Their IARC Classifications (Adapted from Dipple 1985)[90]



Labeled in red are the IARC classifications of the PAHs.

Group **1**: Known to cause cancer in humans

Group **2A**: Probably carcinogenic to humans

Group **2B**: Possibly carcinogenic to humans

Group **3**: Not classifiable as to its carcinogenicity to humans

Absorption, Distribution, Metabolism and Excretion of PAHs in Humans

PAHs can be absorbed into the human body by inhalation, ingestion or dermal absorption.[80] The rate of absorption after inhalation depends on the properties and sizes of the particle carriers. Pure PAHs are cleared by 50% from the lung within 5 hours, while PAHs attached to small carbon particles take around 36 hours to be cleared by 50% from the lung in mice.[91] PAHs adsorbed to water-soluble particles are easily dissolved and absorbed by mucoepithelial cells.[92] More than 95% of pure PAHs are cleared within 24 hours after inhalation.[92] PAHs are absorbed at alveoli, enter the blood circulation, and then are metabolized by cytochrome P-450 enzymes in the liver. A study by Withey et al showed that the distribution of benzo[a]pyrene is highest in lung and fat at 0 and 6

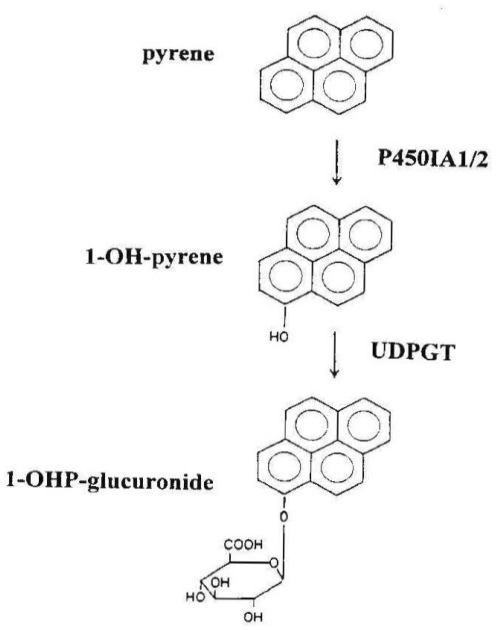
hours, respectively after the inhalation.[93] Immediately after inhalation, the levels of benzo[a]pyrene and total PAH metabolites in organs were as follows: lung>blood>liver>kidney>fat>fetus. While at 6 hours, the order was fat>lung>kidney>liver>blood>fetus.

A common exposure pathway in humans is ingestion of food containing PAHs. A large proportion (38-58%) of PAHs in ingested food is absorbed.[80, 94] Benzo[a]pyrene, pyrene, chrysene and anthracene are easily absorbed by the gastrointestinal tract (50-80%), while phenanthrene is poorly absorbed (4-7%).[80, 94] PAH absorption in the intestines is enhanced by bile acid produced by the gall bladder.[95] Dermal absorption is another common absorption pathway for PAHs. Dermal absorption of benzo[a]pyrene and pyrene from coal tar treatment is about 25% and 20% on human skin, respectively.[96] PAH absorption also varies by anatomical site in the order: shoulder> forehead, forearm, groin > ankle, hand (palmar site).[97] Few studies have examined the detailed distribution of PAHs by ingestion and dermal absorption. However, a study in rats found that 3 hours after ingestion, the levels of benzo[a]pyrene and its metabolites were highest in lung, kidney and liver.[98] For the dermal route, a study in rats found that the level of pyrene and metabolites was highest in liver, kidneys and fat.[99] The metabolism of PAHs occurs in several organs, including the liver, lung and kidneys.[80]

Metabolites of PAHs in serum and urine have been used extensively in various studies as internal dose biomarkers to quantify PAH intake by participants. Hydroxylated metabolites and DNA and protein adducts have been used in both occupational and non-occupational studies to quantify the internal dose of PAHs.[100-102] However, the use of DNA and protein adducts of PAHs is somewhat limited due to cost, detection limits, and stability issues, especially in frozen stored urine.[100] Therefore, hydroxylated metabolites are candidate options for our current study involving the analysis of 1,343 human urine samples. In particular, the metabolites of pyrene, 1-hydroxypyrene (1-OHP) and 1-hydroxypyrene-glucuronide (1-OHPG) (Figure 2.13) are widely used as surrogate internal dose biomarkers in many studies including the large US cross-sectional study, NHANES, and several other studies.[100, 101, 103, 104] In addition, in prior investigations, Dr.

Strickland's laboratory has used biomarkers of PAH exposure in a variety of occupationally or environmentally exposed populations.[101, 103] Urinary 1-OHPG, the major human metabolite of pyrene, or its deconjugated form 1-OHP, have been shown to be elevated in smokers, patients receiving coal tar treatment, asphalt road pavers, and coke oven and blast furnace workers, and subjects ingesting broiled meat.[100, 103, 105]

Figure 2.13: Metabolism of Pyrene and Formation of 1-OHPG



Dr. Strickland's study of steel plant workers in Korea showed exposure-related increases in the concentration of 1-OHPG in post-shift urine samples.[103] In the current dissertation, the selected internal dose biomarker of PAHs will be 1-OHPG. Since the half-life of urinary 1-OHPG is about 6-35 hrs,[106] the levels of 1-OHPG will reflect the PAHs that cleanup workers were exposed to on the day of urine collection or the cumulative dose from the day before. Levels of 1-OHPG can be roughly classified as high, medium and low based on occupational exposures (Table 2.5).

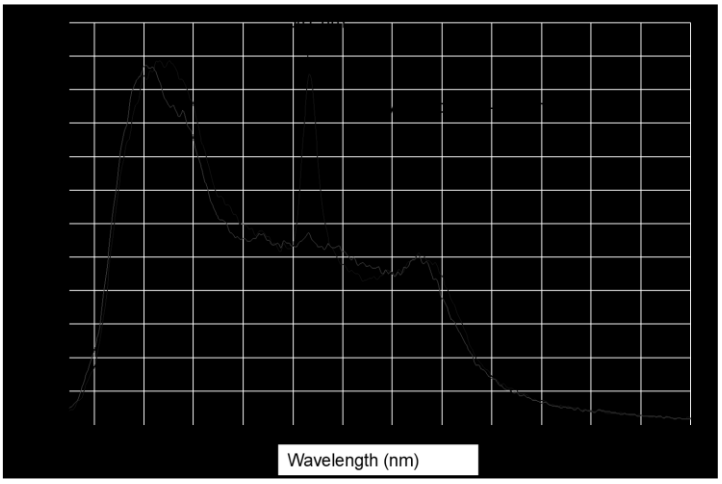
Table 2.5: Levels of PAH Exposure by Urinary 1-OHPG Concentration (pmol/ml) Among Steel Plant Workers (Adapted from Kang et al. 1995)[103]

Levels of Exposure	Steel Plant Jobs	1-OHPG Concentration (pmol/ml) Mean(sd)	
		Smokers	Non-Smokers
High	Blast furnace, coke oven	4.91 (1.02)	2.84 (0.90)
Medium	Casting, steel/iron production	0.94 (0.42)	0.70 (0.28)
Low	Office, construction, transport	0.55 (0.07)	0.26 (0.06)

Laboratory Analysis of 1-OHPG

In this dissertation, urinary 1-OHPG was measured using immunoaffinity chromatography and synchronous fluorescence spectroscopy (SFS), modified from protocols of Strickland et al, 1994.[101] Urine samples (2 ml) were treated with 0.1 N HC1 (at 90°C) for 60 minutes to hydrolyze acid-labile metabolites. The hydrolyzed samples were neutralized and loaded onto Sep-Pak C18 cartridges (Waters), washed with 4 ml of 30% methanol (in water), and the relatively non-polar metabolites were eluted with 4 ml of 80% methanol (in water). The total eluate volume was reduced (0.5 ml) using vacuum evaporation. Immunoaffinity columns were prepared using poly-prep columns (0.8x4 cm) filled with CNBr-activated Sepharose4B (0.8 ml) coupled with monoclonal antibody 8E11, which recognizes PAH metabolites. Then 1.5 ml of 15mM phosphate buffered saline (PBS) was added to the 0.5 ml eluate samples and incubated at 37°C for 30 minutes. The incubated samples were diluted with an additional 2 ml of PBS and loaded on the immunoaffinity columns. After washing the columns with 35% methanol (in PBS), the bound material was eluted with 4 ml of 55% methanol (in PBS) and collected for SFS analysis. Using SFS with a wavelength difference of 34 nm, samples containing 1-OHPG possess a characteristic fluorescence excitation maximum at 347 nm with emission at 381 nm (Figure 2.14). Fluorescence intensity was used to quantify 1-OHPG; the limit of detection was 0.03 pmol/ml urine. The detailed protocol can be found in appendix 2.1.

Figure 2.14: Synchronous Fluorescence Spectroscopy (SFS) of Immunopurified Urine Samples from Subjects with or without Recent Exposure to PAHs



Health Effects of PAHs

In occupational settings, chronic high levels of PAHs can cause bloody vomit, breathing problems, chest pains, chest and throat irritation, and abnormalities in chest X-rays.[107] In the general population, people exposed to PAHs from traffic can develop increased respiratory symptoms such as cough, wheezing, irritating throat, exacerbation of asthma, allergic rhinitis and impaired lung function.[104, 108-111] In addition, two studies in oil spill cleanup workers, reported that urinary metabolites of VOCs or PAHs were also related to increased prevalence of respiratory symptoms.[46, 112]

PAHs are suspected to play a role in smoking-induced atherosclerosis and other cardiovascular effects.[113-116] One hypothesis is that the PAHs can down-regulate the liver X receptor (LXR) alpha gene that controls cholesterol distribution in humans. This may stimulate cholesterol deposition in the endothelial cells of blood vessels.[114] In addition, studies using NHANES data found that urinary metabolites of PAHs were related to self-reported cardiovascular diseases and biomarkers of inflammation related to cardiovascular diseases.[117-119] The carcinogenic effects of PAHs are well documented**.** Studies in humans and animals demonstrate the genotoxicity of PAHs by epoxide formation.[89, 120-123] The most common cancer related to PAHs is lung cancer,[124-126] while other cancers associated with PAHs are stomach, esophageal and skin cancer.[80]

Factors Associated with PAH Exposure in Oil Spill Cleanup Workers

PAHs are a component of crude oil, and factors related to PAH exposure among oil spill cleanup jobs are expected to be: day of cleanup work, duration of cleanup work and job descriptions. PAHs in crude oil can last for up to weeks or months, depending on the specific PAH. If cleanup procedures are effective, then the amount of crude oil (and with the amount of PAHs) at the spill site should decrease as the cleanup progresses. Workers who participate in the first week of cleanup should be exposed to higher levels of PAHs than the workers who work in subsequent weeks. Regarding specific job descriptions, workers whose jobs involve direct contact with crude oil, including oil dispersant spraying, and shoveling and removal of oil contaminated rock and sand, should have higher levels of PAH exposure than support workers whose jobs do not involve direct contact with oil, such as supervisors, journalists, photographers and health care workers. Similarly, longer duration of work in direct contact with oil should be associated with higher levels of PAH exposure.

Apart from PAHs in crude oil, cleanup workers may be exposed to PAHs from smoking or diet. Cigarette smoke contains various PAHs including benzo[a]pyrene and pyrene in both mainstream and sidestream fractions.[127] The concentration of benzo[a]pyrene in mainstream smoke is reported to be 118-374 ng/cigarette, with the concentration in sidestream smoke estimated to be 2.5 to 10 times higher.[127] Thus both current smokers and non-smokers exposed to secondhand smoke are exposed to PAHs.[104] In current smokers, the levels of 1-OHPG measured in urine are 2-3 fold higher than in the urine of non-smokers.[103]

Diet is another major source of PAH exposure in humans. Foods cooked directly over an open flame or broiled at high temperature causing the pyrolysis of fats, carbohydrates, or proteins, can produce high levels of PAHs.[128] Broiled meat and other smoked foods can contain up to 184 ng BaP/g, and its consumption can increase levels of PAH metabolites in urine.[111,129, 130] Thus, ingestion of smoked or broiled foods by workers may contribute to overall urinary 1-OHPG levels.

2. Benzene

Benzene or benzol is a colorless liquid with sweet odor, highly flammable, water-soluble and volatile.[76] Common sources of benzene exposure in the general population are cigarette smoke[131-133], evaporation from gasoline service stations[134, 135], crude oil, and burning of gasoline, coal and oil.[136, 137] Benzene in air breaks down and reacts with other chemicals within a few days,[138] however, when dissolved in water or soil, benzene breaks down slowly over the course of a few weeks.[139] Therefore, during an oil spill cleanup, workers might be exposed initially to benzene by inhalation in the first few days, but exposed to benzene by dermal absorption in subsequent days and weeks.

Absorption, Distribution, Metabolism and Excretion of Benzene

Benzene can be absorbed into the human body by inhalation, ingestion and dermal absorption. Inhalation is the major route of human exposure to benzene. Immediately after inhalation (5 minutes), 70-80% of benzene is absorbed to the systemic circulation.[140,141] Lindstrom et al. reported that showering with benzene contaminated water resulted in 40% absorption of benzene by inhalation and 60% by dermal absorption.[142]

Information on oral absorption of benzene in humans is usually associated with accidental or intentional poisoning.[143] After ingestion, benzene is rapidly absorbed by the gastrointestinal tract.[143] Data from animal studies indicates that after ingestion, the absorption is 90-97% of the ingested dose.[144, 145] Conversely, benzene is absorbed through the skin to the systemic circulation by passive diffusion. Thus, the proportion absorbed by skin is small, about 0.2-1.1% of the total amount in contact with the skin, as reported in various human studies.[146-148]

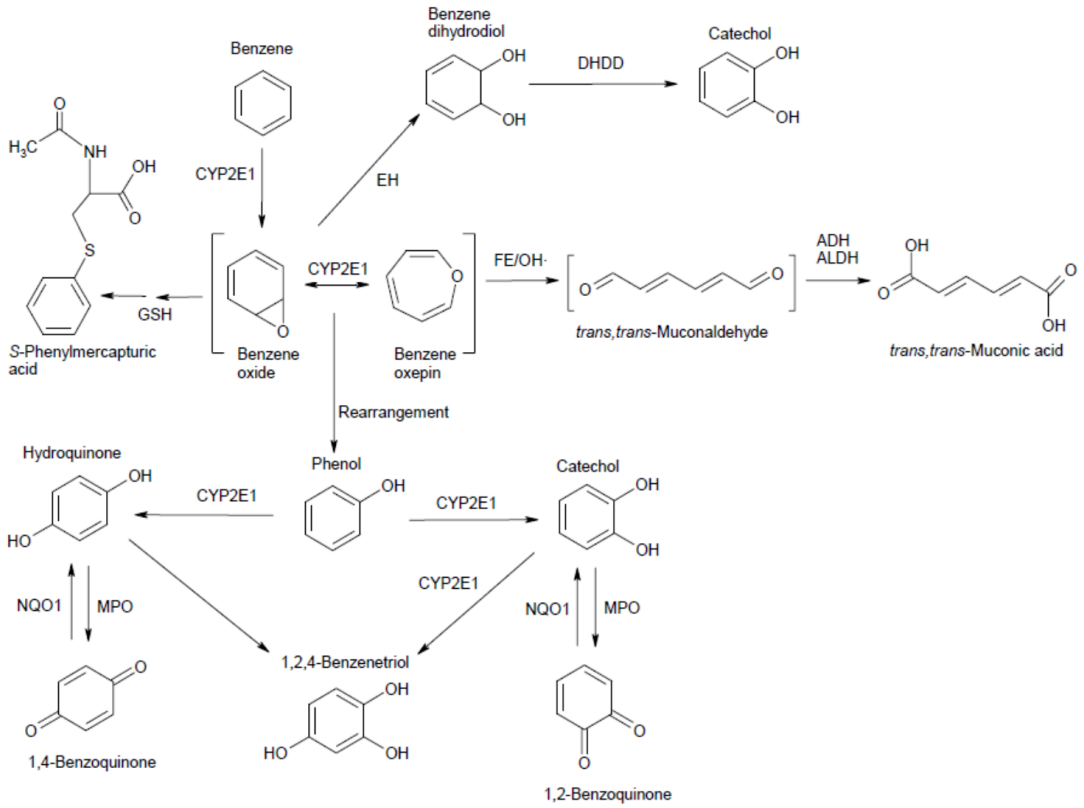
Since benzene is lipophilic, its distribution to fatty tissues is expected. After inhalation, ingestion or dermal absorption, benzene is distributed throughout the body by blood. Autopsies of dead workers who inhaled high levels of benzene showed that organ levels of benzene ordered by concentration were: 3.9 mg% in brain, 2.2 mg% in abdominal fat, 2.0 mg% in blood, 1.9 mg% kidney, 1.6 mg% in liver and 1 mg% in stomach.[76] Benzene is rapidly metabolized to catechol[149]

and hydroquinone, a genotoxic metabolite.[150] In rodent studies, six hours after inhalation, benzene was found in blood, bone marrow, fat, kidney, lung, liver, brain and spleen. However, its metabolites, phenol, catechol and hydroquinone were found mainly in bone marrow at levels higher than in blood.[150, 151]

Metabolism

The first step of benzene metabolism takes place in the liver, where benzene is biotransformed by the phase I enzyme cytochrome P-450 (CYP) 2E1 via oxidative reaction forming benzene oxide.[152] There are various oxidative reactions and pathways that benzene oxide can undergo (Figure 2.15). The predominant pathway is to form phenol by non-enzymatic rearrangement.[153] Phenol is then oxidized by CYP2E1 to catechol or hydroquinone, which are further oxidized by myeloperoxidase to the reactive metabolites 1,2- and 1,4-benzoquinone, respectively.[154] Alternatively, benzene oxide may be converted to benzene dihydrodiol and catechol by epoxide hydrolase and dihydrodiol dehydrogenase, respectively.[154] All metabolites, including phenol, catechol, hydroquinone, and 1,2,4-benzenetriol can undergo conjugation with glucuronic acid or sulfonyl groups.[154] Alternatively, benzene oxide can react directly with glutathione to form S-phenylmercapturic acid (S-PMA) or undergo iron-catalyzed ring-opening to form trans,trans-muconic acid (t,t-MA).[144, 154] The metabolic pathways of benzene are summarized in figure 2.15.

Figure 2.15: Metabolic Pathways for Benzene (Adapted from Nebert et al. 2002)[76, 154]



ADH = Alcohol Dehydrogenase; ALDH = Aldehyde Dehydrogenase,
CYP2E1 = Cytochrome P-450 2E1, DHDD= Dihydrodiol Dehydrogenase; EH= Epoxide Hydrolase; GSH = Glutathione; MPO = Myeloperoxidase;
NQ01= NAD(P)H Quinone Oxidoreductase

The major elimination pathway of benzene is via exhalation. The exhalation of benzene has three phases, starting with a rapid phase (half-life =42 min) followed by a slower phase (half-life = 8 hours).[155] A smaller proportion of absorbed benzene is excreted in urine (0.2%) as phenol and conjugates formed with glucuronides and sulfates.[76,148]

Urinary Biomarkers of Benzene

Urinary metabolites of benzene are important internal dose biomarkers. Three urinary metabolites of benzene that have been extensively used as biomarkers in occupational settings are urinary phenol, t,t-MA and S-PMA.[156-158] Each of these metabolite has its own utility, strengths and drawbacks. Urinary phenol correlates well with benzene at high concentrations -- levels as

high as 620 ppm,[159] however it is insensitive at levels of benzene less than 1 ppm.[158] Phenol is also not specific to benzene because intake of certain drugs or medicines such as phenyl salicylate can raise levels of phenol.[160] t,t-MA and S-PMA are more sensitive than phenol, especially at benzene levels less than 1 ppm.[52, 161] The half-life of t,t-MA (5 hours) is somewhat shorter than that of S-PMA (8-9 hours).[52, 161] More importantly, t,t-MA can be confounded by sorbic acid which is found in processed foods including ham, wine and citrus fruit juices.[162] Overall, S-PMA is more specific to benzene than t,t-MA.

Two methods are commonly used to measure t,t-MA: high-performance liquid chromatography (HPLC) and gas chromatography (GC). The limit of detection for both methods is usually about 10 ng/ml.[158] The HPLC method was used by the Rayong hospital to measure urinary t,t-MA in the Rayong oil spill cleanup workers,[163] however, the limit of detection of these measurements is not specified.

Health Effects of Benzene

Acute toxicity has been reported in individuals exposed to high levels of benzene. In extreme cases, individuals exposed to benzene at levels as high as of 20,000 ppm experienced rapid death by asphyxiation, central nervous system depression and cardiac collapse.[164] Autopsy of dead workers accidentally exposed to high levels of benzene revealed acute granular tracheitis, laryngitis, bronchitis, and massive hemorrhage in lung.[149] Less extreme chronic exposure to benzene has been studied in occupational settings. Nasal irritation, dyspnea, and sore throat were reported in workers exposed to benzene concentrations of more than 60 ppm for more than 1 year.[165, 166]

Acute hematologic effects have been reported in workers exposed to benzene at levels more than 60 ppm. Leukopenia, anemia and thrombocytopenia were observed in these workers more than 2 days after exposure.[167] At levels of benzene below 1 ppm, hematologic effects have also been observed. Lan et al reported that most types of white blood cells decreased in workers exposed to <1 ppm benzene, except for monocytes, CD8+-T cells, and platelets.[168] Hemoglobin decreased

only among the highest exposure group (>10 ppm) after adjusting for age, gender, cigarette smoking, alcohol consumption, recent infection, and body mass index.[168] Over longer time periods, sub-chronic to chronic exposure to benzene can result in pancytopenia, aplastic anemia and eventually leukemia -- especially acute myelogenous leukemia (AML).[76, 169-175]

Factor Associated with Benzene Exposure in Oil Spill Cleanup Workers

The main source of benzene in an oil spill is directly from the crude oil. Therefore, factors associated with potential benzene exposure are day of work, duration of work, and job description. Benzene is volatile and can break down in air within a few days, and in water within a few weeks. Consequently, benzene levels would be expected to decrease fairly rapidly within the first few days of cleanup. Benzene metabolite levels in workers should be high in the first few days of cleanup, then rapidly decline in subsequent weeks. Regarding job descriptions, workers with direct contact with crude oil are expected to have higher levels of benzene exposure than those with jobs that do not involve direct contact with oil, such as supervisors, reporters, photographers, and health care workers. Similarly, longer duration of cleanup work should be associated with higher levels of benzene exposure.

Another factor that will confound benzene biomarker measurements is cigarette smoke. The average heavy smoker (32 cigarettes per day) takes in about 1.8 mg of benzene per day.[80] This is estimated to raise the mean urinary concentration of urinary t,t-MA in smokers 3- to 5-fold compared to the mean concentration in nonsmokers.[176] This implies that cigarette smoking may affect the urinary level of benzene metabolites to a greater degree than PAH metabolites.

Finally, sorbic acid in processed meat, wine, and citrus fruit juice can interfere with and bias the results of urinary t,t-MA measurements, leading to misclassification or overestimation of benzene exposure.[162]

CHAPTER 3: EXPOSURE ASSESSMENT OF RAYONG OIL SPILL CLEANUP WORKERS

ABSTRACT

Background: In July of 2013, a pipeline connecting an offshore oil platform to a tanker caused crude oil to spill into the Sea of Rayong off the coast of Thailand. The estimated amount of oil spilled was between 50 and 190 cubic meters or 336-1,200 barrels. The resulting oil slick washed ashore one day later on the island of Samet. On-land cleanup lasted about a month and was performed by a combination of territorial defense volunteers, citizen volunteers, Thai military personnel and company employees. We conducted a study to quantify internal dose of polycyclic aromatic hydrocarbons (PAHs) and benzene in these workers and to examine factors related to their dose.

Methods: Frozen stored urine samples (n=1343) collected from the workers throughout the one month cleanup were used to measure the concentration of 1-hydroxypyrene-glucuronide (1-OHPG), cotinine and creatinine. Data from questionnaires and urinary trans,trans-muconic acid (t,t-MA) measured as part of a cleanup worker health surveillance plan, were linked with the laboratory data.

Results: The internal dose of PAHs as measured by urinary 1-OHPG was highest in individuals who worked during the first 3 days of cleanup work (median: 0.97 pmol/ml) and was 66.7% lower (median: 0.32 pmol/ml) among individuals who worked in the final week of the study (days 21-28). This was consistent with our hypothesis that the exposure levels of PAHs would be the highest in the first week of cleanup and decline thereafter. After adjusting for cotinine and creatinine by regression analysis, the decline in urinary 1-OHPG concentration with days of cleanup remained significant (P-trend <0.001). Job descriptions with the highest level of urinary 1-OHPG after adjustment were oil dispersant applicators and contaminated sand/trash handlers. A decreasing trend by days of cleanup was also observed for detectable urinary t,t-MA percentage (P-trend <0.001).

Conclusion: Rayong oil spill cleanup workers exhibited evidence of elevated levels of PAH and benzene exposure during the early weeks of cleanup, compared to near background levels 4 weeks after cleanup began. Long-term health monitoring of oil spill cleanup workers should be implemented.

INTRODUCTION

The frequency and size of off-shore oil spills has increased dramatically in the last 50 years.[1] More than 11,000 oil spill-related publications have been published since 1968.[1] Spilled crude oil can affect the environment, local economics and the health of local communities.[2, 3] A recent medium tier oil spill in the Sea of Rayong resulted in a month-long cleanup effort by a combination of local volunteers, military personnel, and oil company employees. We conducted a study to quantify internal dose of polycyclic aromatic hydrocarbons (PAHs) and benzene in these workers and to examine factors related to their dose.

On 27 July, 2013, a pipeline connecting an offshore oil platform to a tanker, operated by PTT Global Chemical (PTTGC), a corporation owned by the government of Thailand, leaked and caused crude oil to spill into the Sea of Rayong off the coast of Thailand.[4] The crude oil covered an area of approximately 20 square kilometers and washed ashore on the island of Samet in an area called "Ao Prao" on 28 July, 2013.[5] The estimated amount of oil spilled was between 50 and 190 cubic meters or 336-1,200 barrels.[4] On-land cleanup lasted about a month and was performed by a combination of territorial defense volunteers, citizen volunteers, Thai military personnel and PTTGC employees. Cleanup procedures included oil containment, skimming, and dispersal, using absorbent pads, high-pressure water spraying and removal and disposal of contaminated soil, sand and rocks.[5]

Crude oil is a complex mixture of hydrocarbons, some of which may affect human health, including volatile organic compounds (VOCs), such as benzene, and polycyclic aromatic hydrocarbons (PAHs), such as pyrene and benzo[a]pyrene.[6] Chronic exposure to benzene has been associated with acute myelogenous leukemia and acute non-lymphocytic leukemia.[7] Some PAHs, including benzo[a]pyrene, have been associated with lung and liver cancers (adenocarcinoma).[8] Several studies from previous oil spill incidents, including the Hebei Spirit and Deepwater Horizon oil spills, found elevated levels of metabolites of PAHs and VOCs in the urine of cleanup workers.[9-11] Ha et al. (2012) found that a subgroup of the Hebei Spirit oil spill workers had elevated levels

of trans,trans-muconic acid (t,t-MA), a metabolite of benzene; mandelic acid, a metabolite of VOCs; and 1-hydroxypyrene, a metabolite of PAHs, compared to levels before their participation in the cleanup effort (N=105).[9] From the same incident, Cheong et al. (2012) found that levels of urinary 1-hydroxypyrene in cleanup workers in the second and third weeks of cleanup (N=124) were higher than levels in workers in the fifth and sixth weeks of cleanup (N=30).[10]

During and after the Rayong oil spill cleanup, the Rayong Provincial Public Health Office and Rayong Hospital designed a health surveillance plan for the workers, collecting urine samples post-shift to assess urinary ttMA. These samples were analyzed in several government laboratories in Thailand, however, the results were only partially reported as categories (>or<500 ug/gCr),[12] rather than as continuous values. In the current study, we have re-examined these ttMA measurements as continuous data (including values below 500 ug/gCr) and expanded the laboratory analysis to include an internal dose biomarker of PAHs, 1-hydroxypyrene-glucuronide (1-OHPG). These results should expand our understanding of the exposures sustained by these workers and lay the groundwork for further assessment of potential acute and chronic health effects.

MATERIALS AND METHODS

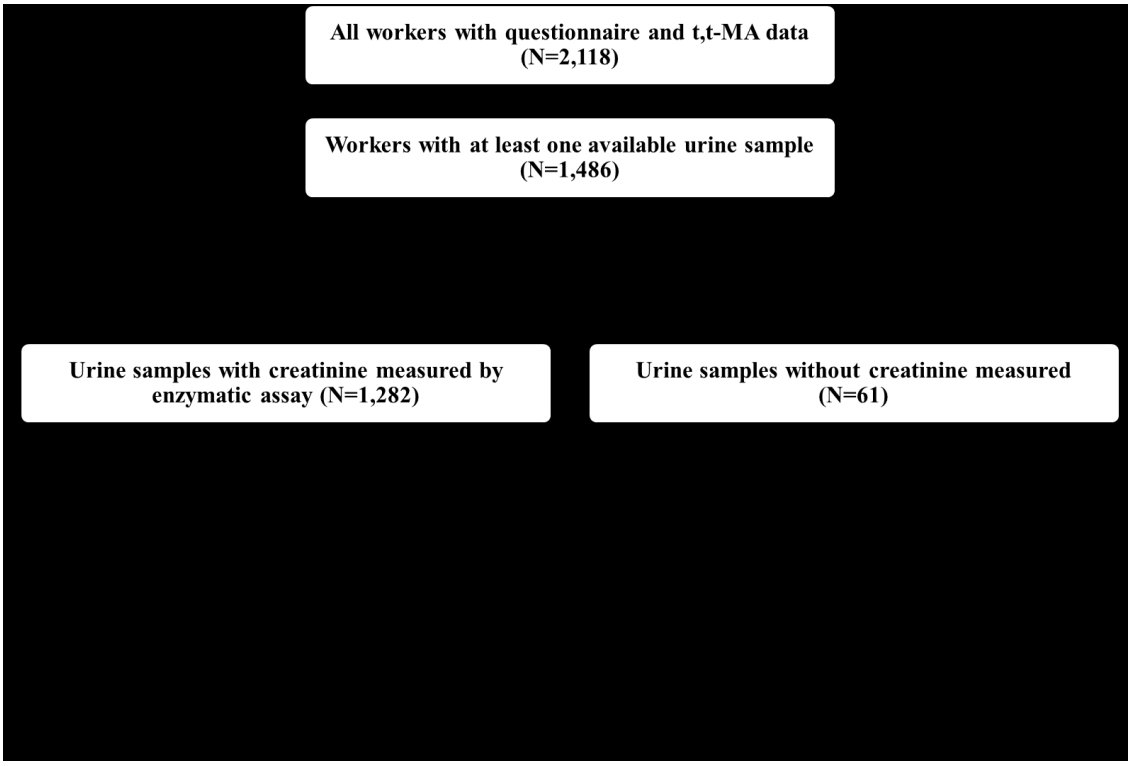
The urine samples were first collected as part of the health surveillance for oil spill cleanup workers. The consent for use of urine samples for scientific study was obtained by the Rayong Hospital and the Thai Naval Medical Department. Approval for the analysis of de-identified urine samples and data in our study was approved by the institutional review board of the Johns Hopkins Bloomberg School of Public Health, and the ethical committees of the Prince of Songkla University, Rayong Hospital, and the Thai Naval Medical Department.

Study Populations and Urine Samples

Our study used the available data and frozen urine samples previously collected by Rayong hospital. The urine samples were transported to our laboratory in Baltimore, MD, USA, on dry ice. The total number of oil spill cleanup workers with available questionnaire and urinary t,t-MA data was 2,118. Of the 1,486 urine samples available to our research team, 1,343 samples had sufficient

volume (≥ 2 mls), for measuring urinary 1-OHPG and cotinine. Creatinine was previously measured in 1,282 of those samples by Rayong Hospital, and we measured creatinine in the remaining 61 urine samples in our laboratory. Figure 3.1 below summarizes our selection process for urine sample analyses.

Figure 3.1: Flow Chart Summarizing Urine Sample Selection and Analyses

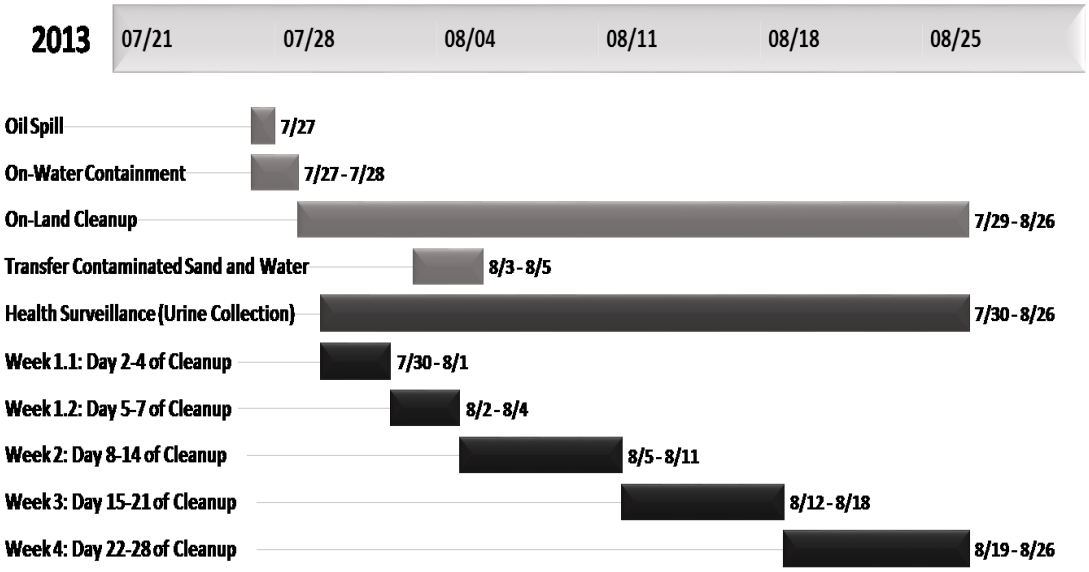


The urinary creatinine measurements previously performed by Rayong Hospital used an enzymatic assay (OSR 61204) using creatinase enzyme on a Beckman Coulter AU analyzer (Beckman Coulter, Inc., Brea, CA), while our laboratory used Jaffe's kinetic reaction (Cayman Chemical Company, Ann Arbor, MI) to measure the remaining 61 urine samples plus the 60 repeat measurements of urines already assayed by Rayong Hospital. Because the two methods gave slightly different results on assays of the same 60 samples, we adjusted the results of the 61 samples assayed in our laboratory to be consistent with the Rayong Hospital sample set (see Lab Methods).

Characteristics, Job Descriptions and Personal Protective Equipment (PPE) Uses of the Rayong Oil Spill Cleanup Workers.

The date that the on-land cleanup began (29 July 2013) was counted as Day 1 of cleanup in our study. The health surveillance protocol, including questionnaire and urine sample collection, began the next day (Day 2). A calendar depicting the cleanup sequence and our study time periods is shown below (Figure 3.2).

Figure 3.2: Rayong Oil Spill Cleanup Study (27 July 2013 – 26 August 2013)



Of the 1,343 urine samples, 80 were collected from workers on their second day of work, and 1 was collected from a worker on his 3rd day of work (Table 3.1). For these workers, at least 4 days had elapsed between different work days. Generalized estimating equations (GEE) were used to account for the potential dependency of these multiple urine samples.

Table 3.1: Multiple Samples from the Same Individuals Collected on Different Days of Cleanup

	Day 2-4	Day 5-7	Week 2	Week 3	Week 4	Total Samples
1st sample collected	537	326	240	78	81	1262
2nd sample collected	0	2	42	36	0	80
3rd sample collected	0	0	0	1	0	1

Demographic factors and their distribution are shown in Table 3.2. Of 1,343 usable urine samples, 93.2% of them were provided by male workers. The median age was 27 years old (Interquartile Range (IQR) = 18.0) and the majority (55.3%) of the urine samples were provided by workers whose background occupation was military personnel. Forty percent of the urine samples were provided on Day 2 to Day 4 of the oil spill cleanup, and 24.4% of the samples were provided on Day 5 to Day 7 of cleanup. Working hours per day was not available in the questionnaires.

Table 3.2: Demographic Factors of Cleanup Workers

Demographic Factors	Descriptions	Number of Workers	Percent
Total		1,343	100.0
Age	Median (1st-3rd Quartiles)	27.0 (22.0-40.0)	
	Unknown Age	9	0.7
Sex	Male	1,252	93.2
	Female	90	6.7
	Missing	1	0.1
Background	Military Personnel	743	55.3
	PTTGC Company Employees	408	30.4
	Citizen Volunteers	183	13.6
	Unknown	9	0.7
Days of Cleanup	Day 2-4	537	40.0
	Day 5-7	328	24.4
	Day 8-14	282	21.0
	Day 15-21	115	8.6
	Day 21-28	81	6.0

The urine samples were provided by workers who performed various oil spill cleanup jobs. Of 1,343 usable urines, 57.9% were provided by workers whose cleanup job was to manually remove oil-contaminated sand, rocks, and trash (Table 3.3), and 23.5% were from workers whose job description was to vacuum or manually remove the oil slick from water.

Table 3.3: Job Descriptions of Cleanup Workers

Job Descriptions	Number of Workers	Percent
Total	1,343	100.0
Contaminated Sand/Trash Removal	778	57.9
Oil Vacuum/Oil Slick Removal	315	23.5
Support Personnel*	61	4.5
Supervisor/Health Care Professional	38	2.8
Transport Driver/Ship Pilot	23	1.7
Oil Dispersant Applicator	17	1.3
Environmental Sampling Personnel	9	0.7
Others	44	3.3
Missing	58	4.3

*** Coordinators, PTTGC Corporate Representatives, Visitors, Photographers, and Journalists were grouped as the support personnel.**

Workers who provided urine samples were also asked about their personal protective equipment (PPE) use. They were asked if they wore any PPE, an N95 mask, an R95 mask, any mask with filter, coveralls, gloves or boots. The mask questions were grouped as "any mask use" if the workers answered "yes" to at least one of the questions, regarding the use of N95, R95 or mask with filter. Most of the workers (84%) self-reported using at least one piece of PPE (either mask, coveralls, gloves or boots) during their shifts (Table 3.4). However, only 16.8% of the workers wore the complete set of PPE, and 31.7% reported that they **"**often**"** wore at least one piece of PPE.

Table 3.4: Personal Protective Equipment Use of Workers

Personal Protective Equipment (PPE) Use	Number of Workers	Percent
Total	1,343	100.0
Any PPE Use	1132	84.3
Mask*	603	44.9
Coveralls	523	38.9
Gloves	770	57.3
Boots	589	43.9
Missing Data	49	3.6
Complete Set of PPE (Wearing 4 of The Above)	226	16.8
Frequency of PPE Use		
- Never	46	3.4
- Sometimes	737	54.9
- Often	426	31.7
Missing Data	134	9.9

*Either one of N95, R95 or mask with filter

Laboratory Methods

Urinary 1-Hydroxypyrene-Glucuronide (1-OHPG) Analysis

To quantify the PAH exposure in cleanup workers, 1-OHPG, a metabolite of pyrene measurable in urine, was used as the surrogate biomarker for the whole group of PAHs. Urinary 1-OHPG were measured using immunoaffinity chromatography and synchronous fluorescence spectroscopy (SFS), modified from protocols of Strickland et al.[13] Two mls of urine was hot acid-hydrolyzed with 0.1 NHCl at 90ºC for 1 h. Then, the hydrolyzed samples were loaded onto Sep-Pak C18 cartridges (Waters, Milford, MA, USA) and washed with 4 ml of 30% methanol (in water). The metabolites were then eluted with 4 ml of 80% methanol (in water). The total volume was reduced to 0.5 ml under vacuum centrifugation and phosphate buffered saline (PBS) (pH 7.4) was added to a final volume of 4 ml at 37º Celsius. The samples were then loaded on the immunoaffinity columns, prepared with CNBr-activated Sepharose4B (0.8 ml) coupled with monoclonal antibody

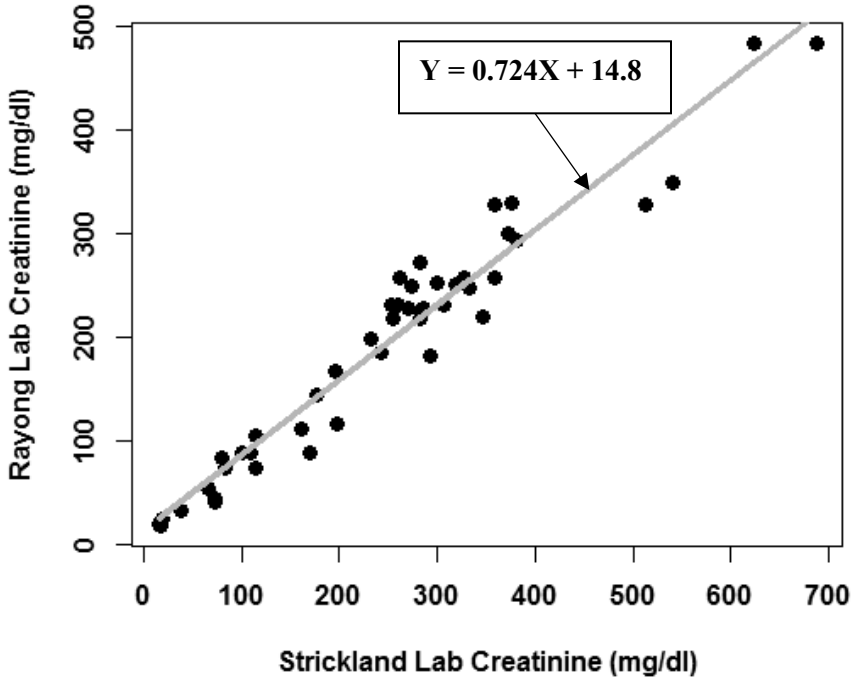
8E11, which recognizes PAHs metabolites. The urinary 1-OHPG fractions were eluted with 55% methanol (in PBS; 4 ml) and collected for synchronous fluorescence spectroscopy analysis (Perkin Elmer LS50B Luminescence spectrometer, Norwalk, CT, USA) using a wavelength difference of 34 nm. Samples containing 1-OHPG possess a characteristic fluorescence excitation maximum at 347 nm with emission at 381 nm. The limit of detection (LOD) was 0.04 pmol/ml. The recovery of the assay was 82% and the coefficient of variation was 5.6%.

Urinary Creatinine Analysis

As mentioned above, 61 urine samples did not have available urinary creatinine data from Rayong Hospital. Therefore, we randomly selected 60 urine samples with available urinary creatinine measurements from Rayong Hosptial as a validation set to compare and quantify the differences in the urinary creatinine levels measured by our laboratory and Rayong Hospital's laboratory. Our laboratory used an assay based on Jaffe's kinetic reaction (Cayman Chemical Company, Ann Arbor, MI) in which a yellow/orange color appears from the reaction between creatinine and alkaline picrate. The color is then extinguished by adding a mixture of sulfuric and acetic acid solution, and the difference in color intensity measured at 490-500 nm before and after acidification is proportional to the creatinine concentration. The coefficient of variation was 5% and the limit of detection was 0.1 mg/dl. Rayong Hospital uses the enzymatic assay (OSR 61204), using creatinase enzyme on a Beckman Coulter AU analyzer (Beckman Coulter, Inc., Brea, CA).

The differences between creatinine from our laboratory and Rayong hospital's laboratory were assessed using linear regression analysis. The coefficient and intercept from the linear regression model were used to calculate and to convert the creatinine concentrations (mg/dl) measured in our laboratory to that of Rayong Hospital's laboratory (Figure 3.3).

Figure 3.3: Scatter Plot Illustrating Differences in Urinary Creatinine Levels between Strickland's (X-axis) and Rayong's (Y-axis) Laboratory



From linear regression, the derived equation was

Y (Rayong's Creatinine) = 0.724*X (Strickland's Creatinine) + 14.8; (r^2=0.94)

We converted Strickland's (our) creatinine to Rayong equivalent concentration before further statistical analysis.

Urinary Cotinine Analysis

Many people are exposed to nicotine from tobacco products either directly or indirectly.[14] Since tobacco smoke is a source of PAHs independent of oil exposure, we measured urinary cotinine, a metabolite of nicotine, to estimate tobacco smoke exposure.[15] In epidemiological research, cotinine in blood, saliva and urine have been used extensively as a valid and specific biomarker for smoking.[16] The half-life of cotinine in urine is about 16-19 hours[16, 17] and therefore, reflects recent smoke exposure within the previous day or two. This corresponds roughly to the half-life of urinary t,t-MA and 1-OHPG, the exposure biomarkers used in our study. We used a

solid phase competitive ELISA (Calbiotech, El Cajon, CA) assay to measure urinary cotinine. The coefficient of variation was 8% and the limit of detection was 2 ng/ml. Generally, a cut-off of 50 ng/ml is recommended to differentiate between non-smokers and passive or active smokers.[18, 19]

Urinary t,t-MA Data

Urinary t,t-MA data from Rayong Hospital was retrieved and linked to the questionnaire data. Urinary t,t-MA, from Rayong Hospital was measured using high performance liquid chromatography with fluorescent detection.[20] The limit of quantitation (LOQ) was estimated to be 0.01 mg/dl or 0.10 µg/ml.

Statistical Methods

All available questionnaire data was linked to the 1-OHPG, t,t-MA, and cotinine measurements. Non-detectable measurements of urinary 1-OHPG and cotinine were replaced with the value of the $LOD/2^{1/2}$, assuming log normal distributions. For descriptive analysis, continuous variables, including urinary 1-OHPG, urinary cotinine, and age were presented as median (1^{st}-3^{rd} quartile) values due to non-normal distributions. Categorical variables, such as number of workers by days of cleanup, PPE use, or job description were presented as number (%).

For inferential statistics, log-linear regression models were used to compare the levels of 1-OHPG among days of cleanup (days 2-4, days 5-7, days 8-14, days 15-21 and days 22-28), adjusting for urinary cotinine and/or creatinine. To adjust for workers' dehydration status, creatinine concentration was added as a covariate in the log-linear regression models. Finally, the log-linear regression models were used to compare levels of 1-OHPG among job description categories, adjusting for days of cleanup and cotinine concentration. P-values for trends of the geometric difference ratios were calculated using Rao's score test.[21] Generalized estimating equations (GEE), as described in Liang and Zeger (1986)[22] were used to account for multiple samples from the same workers.

Detailed t,t-MA data was not reported in the previous two published papers from the Rayong oil spill.[12, 23] Therefore, we re-analyzed the complete urinary t,t-MA data set, previously measured by the Rayong Hospital, and adjusted these results with our cotinine measurements. Because of the large proportion of non-detectable samples (67.5%), the urinary t,t-MA data was analyzed as a binary variable (detectable vs non-detectable). In addition, due to the relatively smaller sample size, t,t-MA data from the 3rd and 4th weeks of cleanup were combined before the statistical analysis. To further adjust for smoking, stratification by nonsmokers and smokers (urinary cotinine $\leq$ 50 ng/ml and >50 ng/ml) and logistic regression was used to assess the association between odds of having detectable t,t-MA in urine and days of cleanup, job descriptions and PPE use, adjusting for cotinine and creatinine. All statistical analysis was completed using R version 3.2.4. (R Development Core Team, Vienna, Austria, 2016)

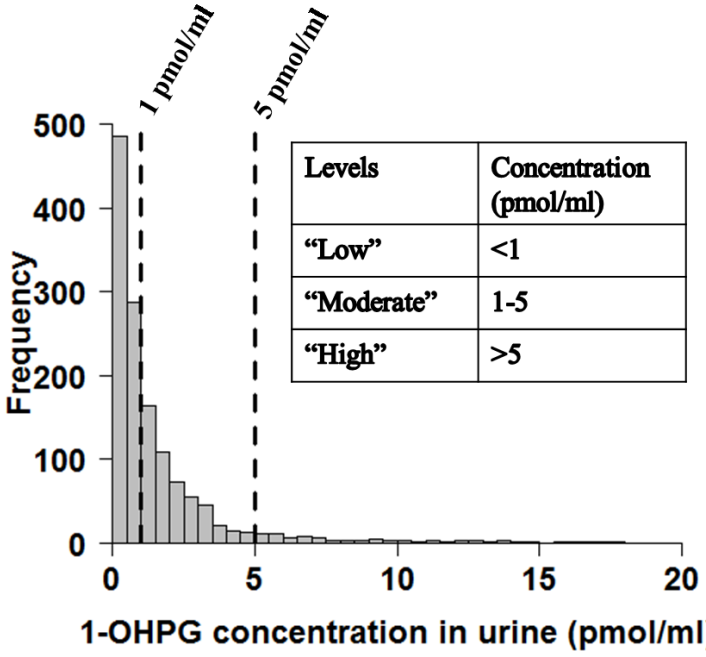
RESULTS

Urinary 1-OHPG

In the 1,343 urine samples analyzed, the median level of urinary 1-OHPG was 0.79 pmol/ml (Q1-Q3: 0.31-1.81). The number of urine samples with the non-detectable levels was 94 (7.0%). Using the suggested categorical values from Kang et al (1995), 57.6% of the urine samples had "low" levels of 1-OHPG (<1.0 pmol/ml), 36.5% had "moderate" levels (1.0-5.0 pmol/ml) and 5.9% had "high" levels (>5.0 pmol/ml) as shown Figure 3.4.

Figure 3.4: Laboratory Results of Urinary 1-OHPG (n=1,343)

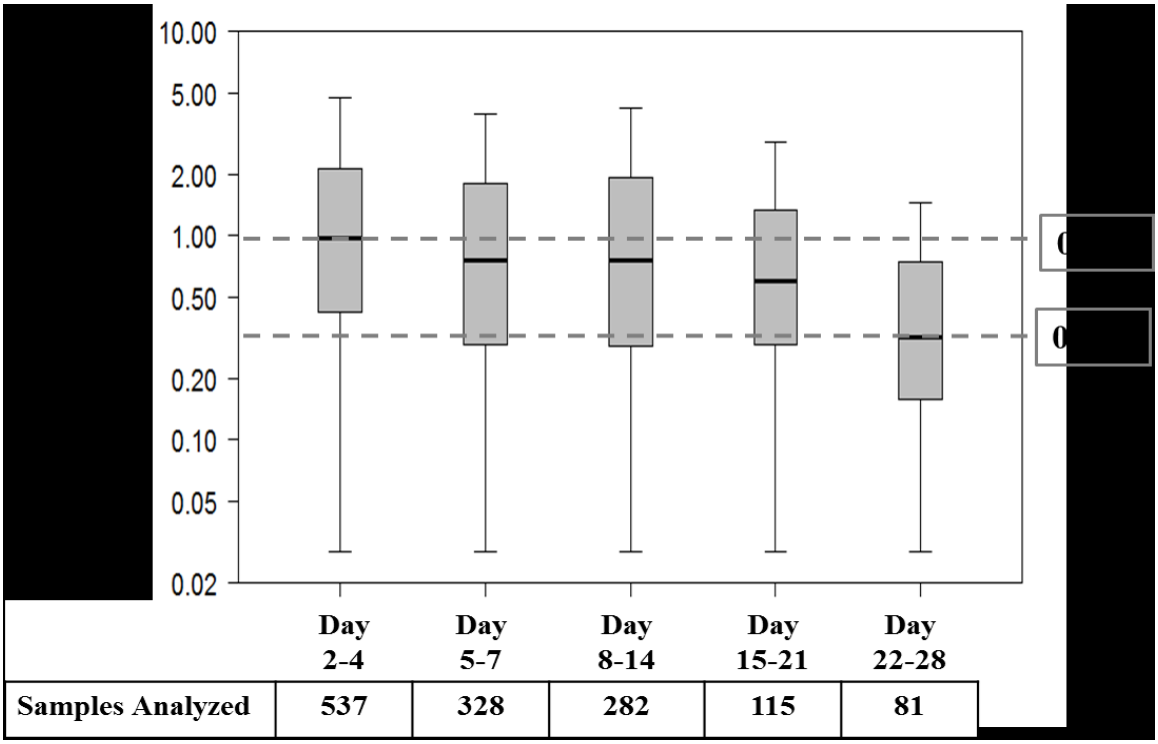
Parameters	Value
Number samples analyzed	1343 samples
Minimum	Non-detectable (n=94)
Q1	0.31 pmol/ml
Median	0.79 pmol/ml
Q3	1.81 pmol/ml
Maximum	78.61 pmol/ml
Non-detectable percentage	7.1%



Levels	Concentration (pmol/ml)
"Low"	<1
"Moderate"	1-5
"High"	>5

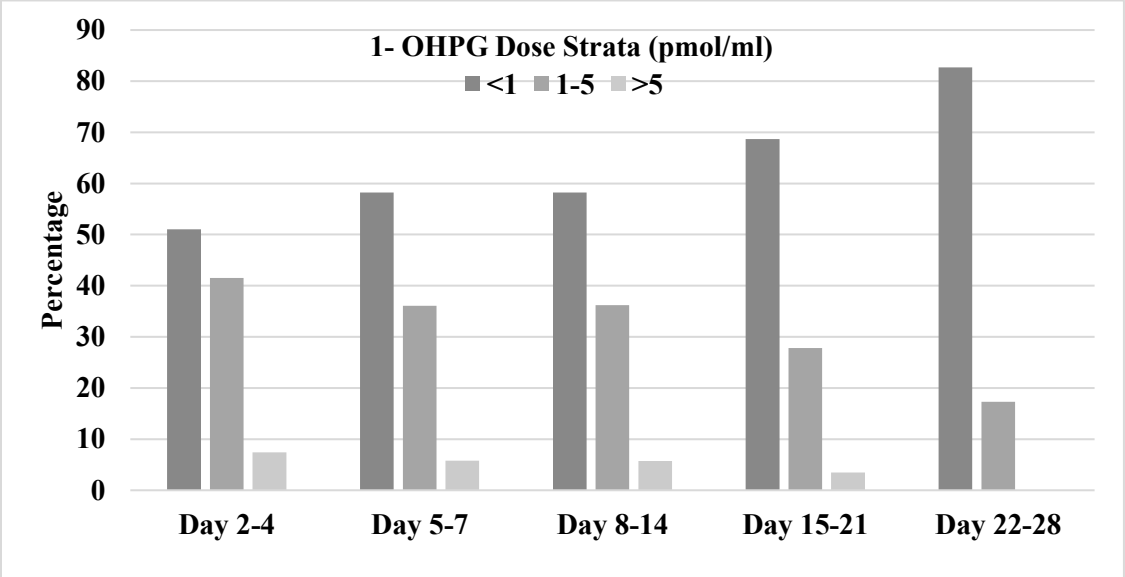
1-OHPG exhibited a decreasing trend by days of cleanup as shown in Figure 3.5. We assigned the starting date of on-land cleanup (29th July) as "day 1 of cleanup" in our study. Urine samples from day 1 of cleanup were not available because the health surveillance protocol was not implemented until day 2 of the study. The median of urinary 1-OHPG on days 2-4 of the Rayong oil spill cleanup was 0.97 pmol/ml, and the levels decreased by 66.7% to 0.32 pmol/ml by day 21-28 of cleanup. This was consistent with our hypothesis that the exposure levels of PAHs would be the highest in the first week of cleanup and decline thereafter.

Figure 3.5: Urinary 1-OHPG (Logscale) by Days of Cleanup



Presenting the urinary 1-OHPG data as dose strata by days of cleanup (Figure 3.6), the proportion of workers with "low" levels of 1-OHPG increased over time from 51.0% on days 2-4 to 82.7% on days 22-28; whereas, the proportion of workers with "high" levels of 1-OHPG decreased over time from 7.4% on day 2-4 to 0% on day 22-28.

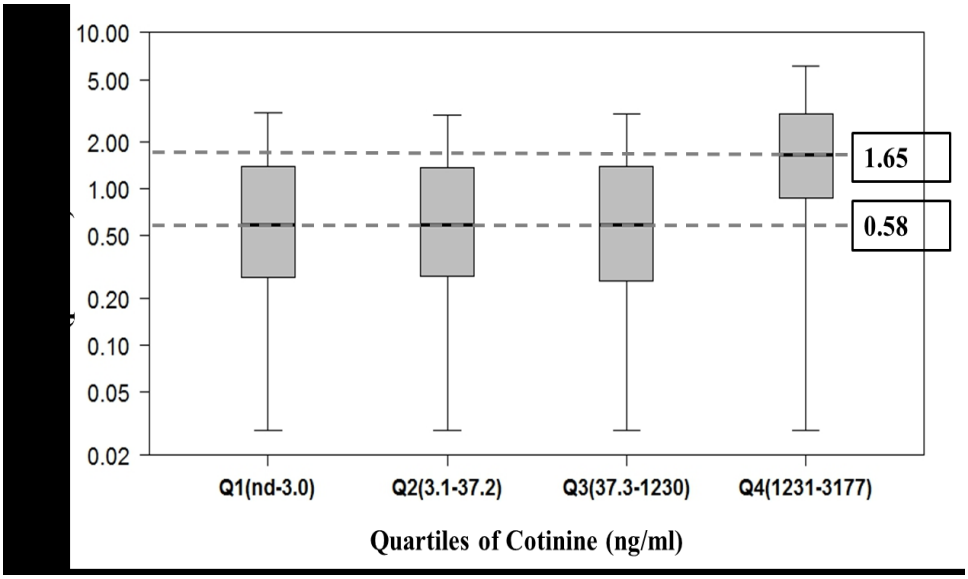
Figure 3.6: Strata of Urinary 1-OHPG by Days of Cleanup



Urinary Cotinine

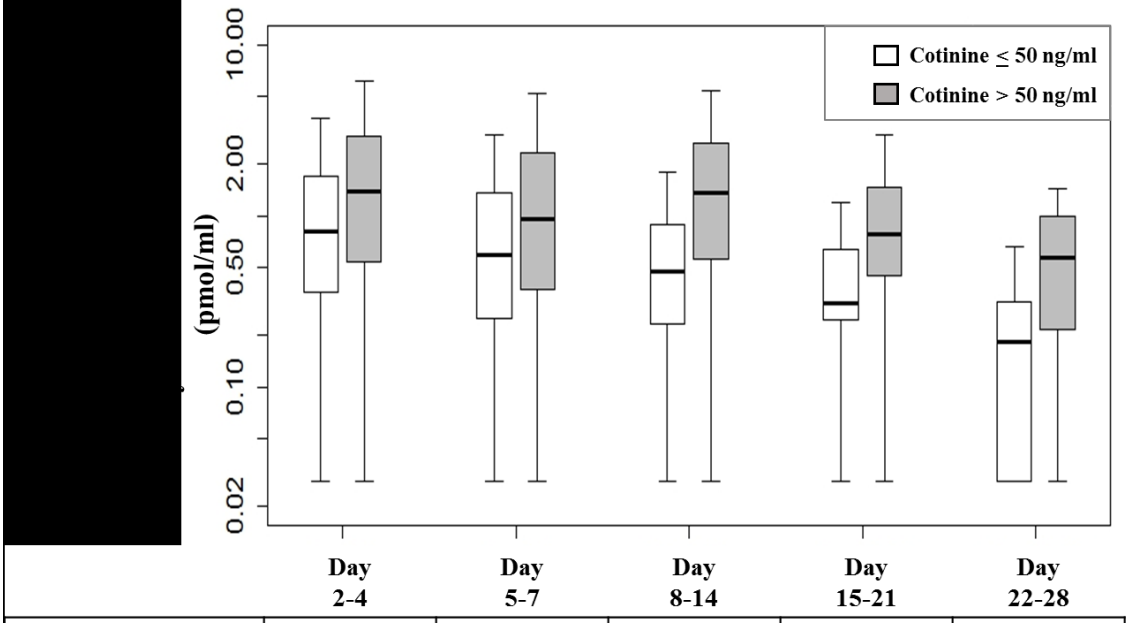
Since smoking status of cleanup workers recorded on questionnaires was limited (only 387 workers had available smoking status) we measured urinary cotinine as a biomarker of tobacco smoke exposure. Overall, the median level of urinary cotinine was 37.3 pmol/ml (Q1-Q3: 3.0-1229.5) and urinary 1-OHPG was 2.8 times higher in the 4th cotinine quartile than in the 1st quartile (1.65 vs 0.58 pmol/ml).

Figure 3.7: Urinary 1-OHPG (Logscale) by Quartiles of Cotinine



Using a urine cotinine cutoff concentration of 50 ng/ml to distinguish between smokers and non-snokers[18, 19], we observed that the median urinary 1-OHPG concentration of smokers was 2 to 3 fold higher than that of nonsmokers, by days of cleanup. In nonsmokers, urinary 1-OHPG exhibited a clearly decreasing trend by days of cleanup, as shown in Figure 3.8. The median concentration of urinary 1-OHPG on days 2-4 in nonsmokers was 0.81 pmol/ml, decreasing by 79% to 0.18 pmol/ml by day 21-28 of cleanup. Whereas in smokers, the median of urinary 1-OHPG on days 2-4 was 1.40 pmol/ml, decreasing by 59% to 0.57 pmol/ml by day 21-28 of cleanup (Figure 3.8).

Figure 3.8: Urinary 1-OHPG (Logscale) by Days of Cleanup in Smokers and Nonsmokers



	Day 2-4	Day 5-7	Day 8-14	Day 15-21	Day 22-28
Cotinine ≤ 50 ng/ml (Nonsmokers)	0.81	0.58	0.47	0.31	0.18
Cotinine > 50 ng/ml (Smokers)	1.40	0.95	1.37	0.78	0.57

Regression Analysis of 1-OHPG by Days of Cleanup.

We performed 3 different log-linear regression models for 1-OHPG and days of cleanup. For Model 1, the association between 1-OHPG and days of cleanup was adjusted solely by urinary creatinine. For model 2, only urinary cotinine was used as an adjusting variable. For model 3, both urinary creatinine and cotinine were used as adjusting variables. All the models showed significantly decreasing trends in 1-OHPG geometric mean (GM) ratio over time (P-Trend <0.001) (Table 3.5). After adjusting for cotinine (Model 2), the GM ratio declines were larger compared to the univariable (unadjusted) model, suggesting that smoking might be a confounder in the association between urinary 1-OHPG and days of cleanup. In Model 3, the declining trend in 1-OHPG GM ratio with days of cleanup remained significant after adjusting for both creatinine and cotinine.

Table 3.5: Log-Linear Regression with GEE* of Urinary 1-OHPG by Days of Cleanup (n = 1,343)

Weeks of Study	Days of Cleanup	Geometric Mean Ratio of 1-OHPG (95% CI)			
		Univariable Model	Model 1	Model 2	Model 3
Week 1.1	Day 2-4	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Week 1.2	Day 5-7	**0.77 (0.64-0.93)**	0.90 (0.77-1.06)	**0.65 (0.54-0.77)**	**0.76 (0.65-0.88)**
Week 2	Day 8-14	**0.80 (0.65-0.98)**	1.02 (0.86-1.22)	**0.64 (0.53-0.77)**	**0.83 (0.70-0.97)**
Week 3	Day 15-21	**0.69 (0.54-0.87)**	0.84 (0.67-1.06)	**0.50 (0.41-0.63)**	**0.62 (0.50-0.77)**
Week 4	Day 22-28	**0.32 (0.23-0.44)**	**0.50 (0.37-0.68)**	**0.27 (0.20-0.36)**	**0.42 (0.32-0.56)**
P-Trend		**<0.001**	**<0.001**	**<0.001**	**<0.001**

Model 1: Adjusted by Urinary Creatinine
Model 2: Adjusted by Urinary Cotinine
Model 3: Adjusted by Urinary Creatinine and cotinine
* **Generalized Estimating Equation with Exchangeable Correlation Structure**
Bold numbers indicate statistically significant results. (P<0.05)

Log-linear regression also showed that urinary cotinine (smoking) and urinary creatinine were independently associated with concentration of urinary 1-OHPG. The GM of urinary 1-OHPG increased by 7% (GM ratio: 1.07, 95% CI: 1.06-1.07) per 100 μg/ml increase in urinary creatinine; and the GM of urinary 1-OHPG increased by 71% (GM ratio: 1.71, 95% CI: 1.57-1.86) per 1 μg/ml increase in urinary cotinine (data not shown).

Urinary 1-OHPG and Job Descriptions

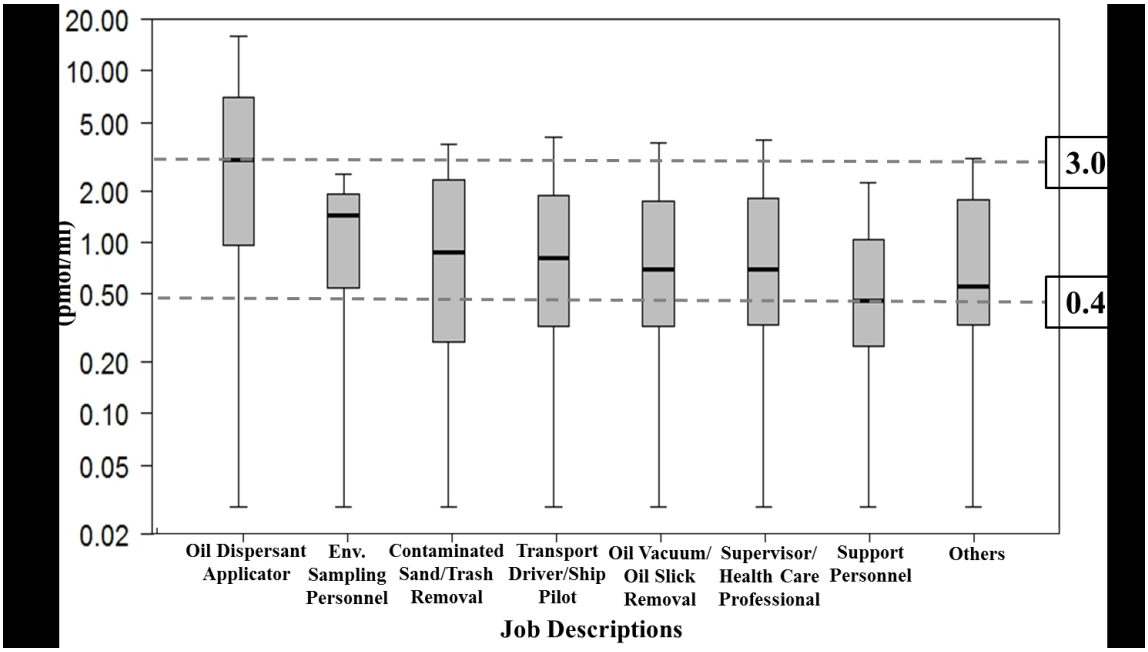
The urinary 1-OHPG levels were stratified by job description of cleanup workers (Table 3.5). Contaminated sand and trash removal (57.9%) was the most common job description. The highest GM level of urinary 1-OHPG was found in urine samples from oil dispersant applicators who sprayed oil dispersants (GM: 1.79, IQR: 0.31-1.81 pmol/ml). The second highest level was found in urine samples from workers who removed contaminated sand and trash (GM: 0.75, IQR: 0.32-1.87 pmol/ml). The lowest 1-OHPG level was found in support personnel (coordinators, PTTGC corporate representatives, visitors, photographers, and journalists) (GM: 0.44, IQR: 0.25-1.04). (Table 3.6 and Figure 3.9)

Table 3.6: Urinary 1-OHPG by Job Descriptions of Cleanup Workers (n = 1,343) (Descending Order by Geometric Mean of 1-OHPG)

Job Descriptions	Urinary 1-OHPG (pmol/ml)				
	Numbers (%)	Geometric Mean	Median	1st Quartile	3rd Quartile
Total	1,343 (100.0%)	0.72	0.79	0.31	1.81
Oil Dispersant Applicator	17 (1.3%)	1.79	3.02	0.97	6.98
Contaminated Sand/ Trash Removal	778 (57.9%)	0.75	0.81	0.32	1.87
Environmental Sampling Personnel	9 (0.7%)	0.72	1.42	0.54	1.91
Oil Vacuum/ Oil Slick Removal	315 (23.5%)	0.70	0.70	0.32	1.72
Supervisor/ Health Care Professional	38 (2.8%)	0.68	0.69	0.35	1.80
Transport Driver/ Ship Pilot	23 (1.7%)	0.61	0.88	0.26	2.31
Support Personnel*	61 (4.5%)	0.44	0.45	0.25	1.04
Others	44 (3.3%)	0.64	0.55	0.34	1.67
Missing	58 (4.3%)	0.72	0.90	0.32	1.66

* **Coordinators, PTTGC Corporate Representatives, Visitors, Photographers, and Journalists were grouped as support personnel.**

Figure 3.9: Urinary 1-OHPG (Logscale) by Job (Descending Order by Median of 1-OHPG)



In the GEE and log-linear regression analysis of 1-OHPG by job descriptions, support personnel, with the lowest GM of urinary 1-OHPG, was used as the reference group (Table 3.7). In the univarible model, compared to support personnel, oil dispersant applicators had the highest 1-OHPG GM ratio (4.1; 95% CI: 1.57-10.69). Contaminated sand/trash removal and oil vacuum/oil slick removal were two other job groups with significantly elevated GM ratios (95% CI) of 1-OHPG, compared to the support personnel (1.71 (1.24-2.36) and 1.61 (1.15-2.67), respectively). The other job groups exhibited non-significantly elevated GM ratios compared to the support (reference) group -- perhaps due to small sample sizes. After adjusting for cotinine (Model 3), the GM ratios (95% CI) of the transport driver/ship pilot group decreased from 1.40 (0.65-3.00) to 1.06 (0.55-2.07), suggesting that this group of workers might include a high proportion of smokers. After adjusting for days of cleanup, urinary creatinine and urinary cotinine (Model 4), only oil dispersant applicators and contaminated sand/trash removal workers demonstrated significantly elevated 1-OHPG, compared to support personnel ((GM ratio: 2.33, 95% CI: 1.29-4.21) and (GM ratio: 1.33, 95% CI: 1.02-1.75), respectively).

Table 3.7: Log-Linear Regression with GEE* of Urinary 1-OHPG by Job Descriptions (n = 1,285)**

Job Descriptions	Geometric Mean Ratio of 1-OHPG (95% CI)				
	Univariable Model	Model 1	Model 2	Model 3	Model 4
Support Personnel[***]	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Oil Dispersant Applicator	**4.10 (1.57-10.69)**	**4.39 (1.68-11.48)**	**3.06 (1.39-6.70)**	**3.20 (1.34-7.64)**	**2.33 (1.13-4.83)**
Contaminated Sand/ Trash Removal	**1.71 (1.24-2.36)**	**1.85 (1.33-2.58)**	**1.56 (1.16-2.10)**	**1.55 (1.14-2.09)**	**1.33 (1.02-1.75)**
Environmental Sampling Personnel	1.65 (0.62-4.36)	1.71 (0.64-4.56)	1.58 (0.70-3.58)	2.08 (0.80-5.46)	1.90 (0.84-4.31)
Oil Vacuum/ Oil Slick Removal	**1.61 (1.15-2.67)**	**1.56 (1.10-2.21)**	**1.37 (1.00-1.86)**	1.36 (0.99-1.87)	1.21 (0.91-1.61)
Supervisor/ Health Care Professional	1.54 (0.94-2.51)	1.58 (0.99-2.54)	1.26 (0.84-1.88)	1.63 (0.99-2.57)	1.31 (0.89-1.92)
Transport Driver/Ship Pilot	1.40 (0.65-3.00)	1.48 (0.68-3.21)	1.55 (0.79-3.03)	1.06 (0.55-2.07)	1.14 (0.66-2.00)
Others	1.46 (0.88-2.43)	1.28 (0.76-2.15)	0.91 (0.58-1.43)	1.14 (0.71-1.84)	0.84 (0.55-1.28)

Model 1: Adjusted by days of cleanup (day 2-4, day 5-7, day 8-14, day 15-21 and day 22-28)
Model 2: Adjusted by days of cleanup and urinary creatinine
Model 3: Adjusted by days of cleanup and urinary cotinine
Model 4: Adjusted by days of cleanup, urinary cotinine and creatinine
* Generalized Estimating Equation with Exchangeable Correlation Structure
** 58 Unknown Job Description
*** Coordinators, PTTGC Corporate Representatives, Visitors, Photographers, and Journalists were grouped as the support personnel.
Bold numbers indicate statistically significant results. (P<0.05)

Urinary 1-OHPG and Protective Equipment (PPE) Use

Personal protective equipment (PPE) use by oil-spill cleanup workers did not show evidence of protection against PAH exposure as measured by urinary 1-OHPG concentration. This was true for overall PPE use, as well as for use of individual equipment, including N95 and R95 masks, gloves, boots and coveralls. The urinary 1-OHPG levels in cleanup workers who wore PPEs, was not significantly lower than in those who did not wear PPEs (Table 3.8 and Appendix Table 3.3). In the univariable model, the GM of 1-OHPG in workers who wore mask or coveralls were higher than the workers who did not wear mask or coveralls ((GM ratio: 1.27, 95% CI: 1.09-1.47) and (GM ratio: 1.40, 95% CI: 1.20-1.63), respectively). This may be because mask and coveralls were used by workers mostly in the early days of cleanup when the 1-OHPG levels were high (data not shown). After adjusting by days of cleanup (Model 1), the GM ratios move toward one (null) (Models 1-3). Although not significant, workers who "sometimes" or "often" used PPE, had lower levels of 1-OHPG, compared to those who never used PPE.

Table 3.8: Log-Linear Regression with GEE* of Urinary 1-OHPG by PPE Use (n=1,294)**

Type of PPE	Geometric Mean Ratio of 1-OHPG (95% CI)			
	Univariable Model	**Model 1**	**Model 2**	**Model 3**
Any PPE Use (vs No PPE use)	0.99 (0.79-1.25)	0.97 (0.77-1.22)	0.97 (0.78-1.20)	0.97 (0.80-1.16)
Mask (vs No Mask use)	**1.27 (1.09-1.47)**	1.12 (0.95-1.31)	1.14 (0.98-1.32)	**1.17 (1.02-1.33)**
Coveralls (vs No Coverall use)	**1.40 (1.20-1.63)**	**1.22 (1.04-1.44)**	1.16 (0.99-1.35)	**1.15 (1.01-1.31)**
Gloves (vs No Glove use)	0.95 (0.82-1.11)	1.05 (0.89-1.23)	1.03 (0.89-1.20)	0.98 (0.86-1.11)
Boots (vs No Boots use)	0.89 (0.77-1.04)	0.95 (0.82-1.11)	0.96 (0.84-1.11)	0.98 (0.87-1.11)
Frequency				
- Never	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
- Sometimes	0.71 (0.47-1.06)	0.76 (0.51-1.13)	0.78 (0.55-1.11)	0.82 (0.60-1.11)
- Often	0.72 (0.48-1.07)	0.75 (0.51-1.11)	0.78 (0.56-1.10)	0.85 (0.63-1.15)

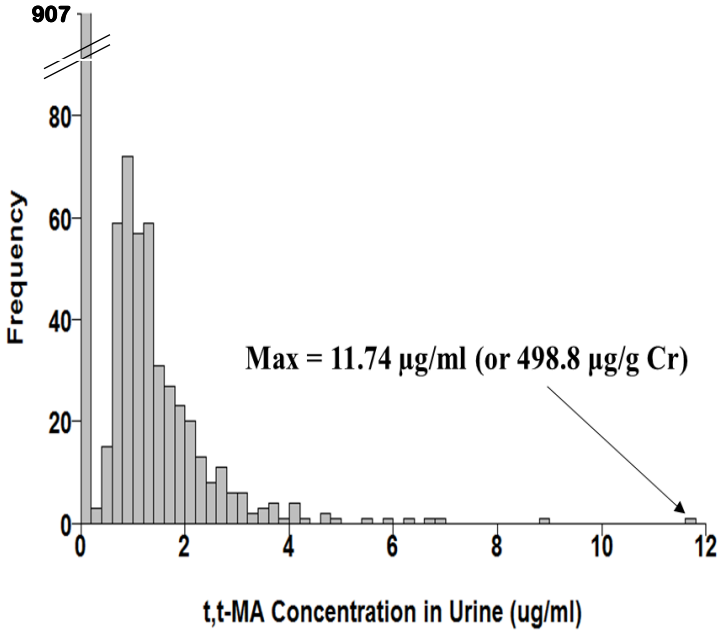
Model 1: Adjusted by days of cleanup (day 2-4, day 5-7, day 8-14, day 15-21 and day 22-28)
Model 2: Adjusted by days of cleanup and urinary cotinine
Model 3: Adjusted by days of cleanup, urinary cotinine and creatinine
*Generalized Estimating Equation with Exchangeable Correlation Structure
**49 workers had at least one piece of missing data
Bold numbers indicate statistically significant results. (P<0.05)

Urinary t,t-MA

The distribution of urinary t,t-MA among cleanup workers is shown in Figure 3.10. There was a large number of samples with non-detectable levels of t,t-MA (907 out of 1,343)(67.5%). The minimum quantifiable concentration, observed from the recorded data was 0.10 µg/ml. Since a large percentage of urine sample had non-detectable levels of t,t-MA, we elected to statistically analyze the t,t-MA data as a binary variable (detectable vs non-detectable). Non-detectable samples were assigned the value LOQ/$2^{1/2}$ or 0.07 ug/ml

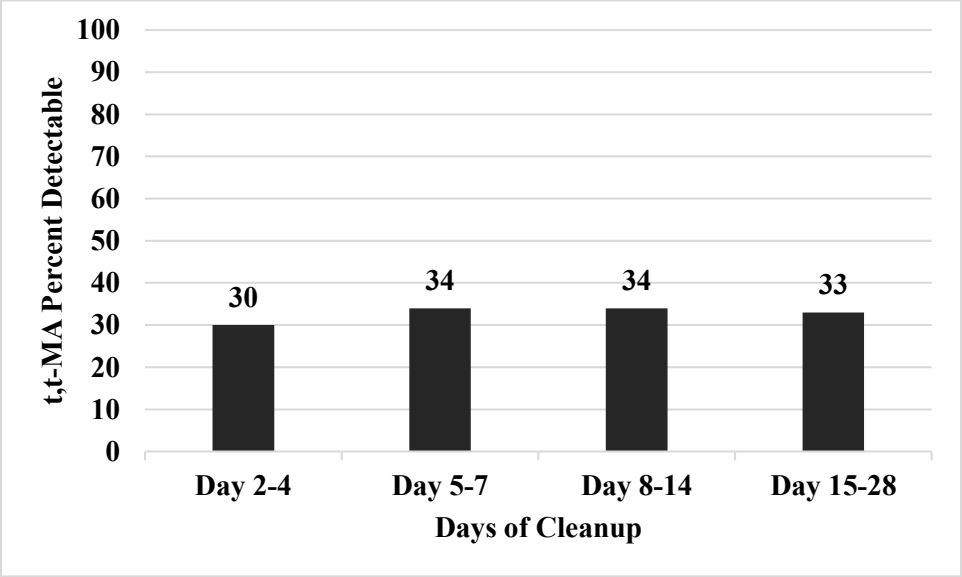
Figure 3.10: Urinary t,t-MA Results from Rayong Hospital

Parameters	Value
Number samples analyzed	1343 samples
Minimum	Non-detectable (n=907)
Q1	0.07 µg/ml
Median	0.07 µg/ml
Q3	0.86 µg/ml
Maximum	11.74 µg/ml
Non-detectable percentage	67.53%



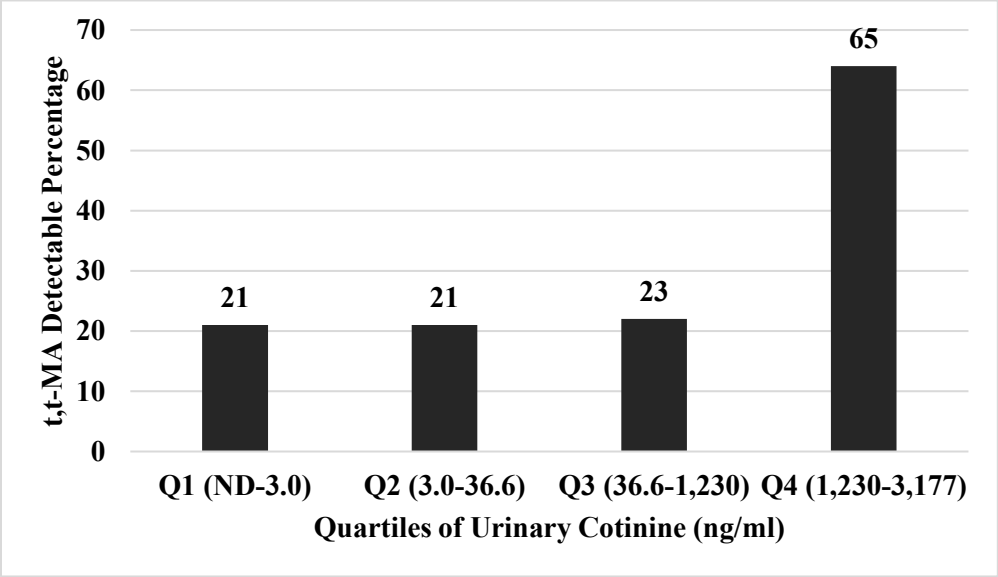
In order to increase statistical power when analyzing t,t-MA as a binary variable, we grouped data from week 3 (day 15-21) and week 4 (day 22-28) together. Before adjusting for covariates, the proportion of urine samples with detectable levels of t,t-MA were not different by days of cleanup (overall t,t-MA detectable percentage = 30-34%) (Figure 3.11).

Figure 3.11: Urinary t,t-MA Detectable Percentages by Days of Cleanup (n = 1,343)



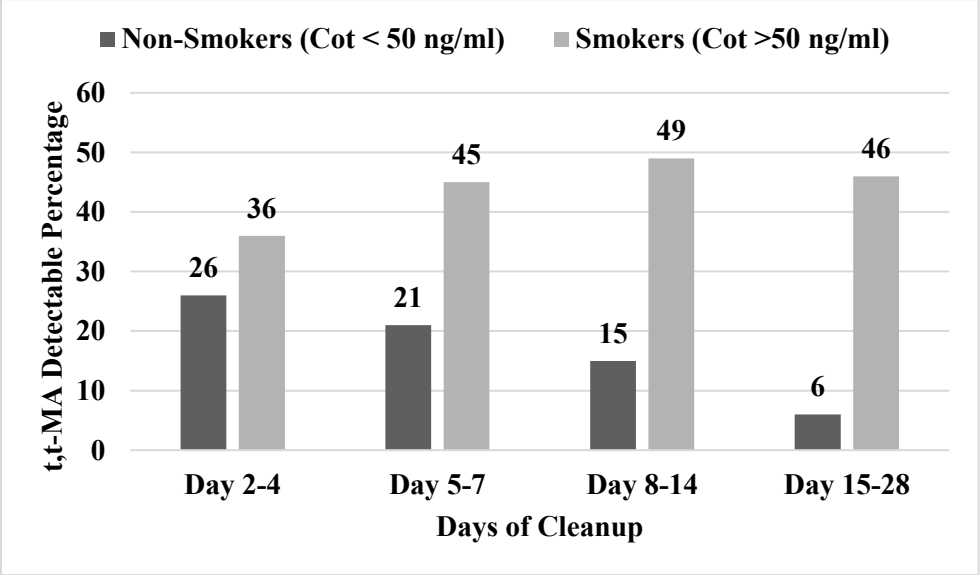
However, this result was confounded by tobacco smoke exposure, which is known to contain benzene. Detectable t,t-MA was more frequent in the urine of smokers (urinary cotinine > 50 ng/ml) than non-smokers (44.2% vs 21.2%, respectively, P<0.001) (Appendix Table 3.2). In addition, by quartiles of urinary cotinine, the percentage of urine samples with detectable t,t-MA was much higher in subjects with cotinine levels in the 4th quartile than in subjects with lower quartiles (64.9% vs 21.3%, 21.1% and 22.6%, P<0.001) (Figure 3.12). Therefore, we controlled for smoking by stratifying urinary cotinine concentrations in subsequent analyses of the association between t,t-MA detectable levels and days of cleanup.

Figure 3.12: Urinary t,t-MA Detectable Percentages by Cotinine Quartiles (n = 1,343)



We distinguished presumed smokers from non-smokers using a urinary cotinine cut-off of 50 ng/ml. The nonsmoker group (cotinine ≤ 50 ng/ml) exhibited a clearly decreasing trend in t,t-MA detectable percentage by days of cleanup (P-trend =0.001) (Figure 3.13). The percentages of non-smoking workers with detectable urinary t,t-MA were 26.3%, 20.9%, 14.8% and 6.3% on days 2-4, days 5-7, days 8-14 and days 15-28 of cleanup, respectively. While in the smoker group (urinary cotinine > 50 ng/ml), a decreasing trend in detectable t,t-MA with days of cleanup was not observed, consistent with our finding that smoking increases the probability of having detectable t,t-MA in urine.

Figure 3.13: Urinary t,t-MA Detectable Percentages by Days of Cleanup in Smokers and Nonsmokers (n = 1,343)



***Smokers were workers whose urinary cotinine was more than 50 ng/ml.**

By logistic regression, the odds ratio of having detectable urinary t,t-MA among nonsmokers (urinary cotinine ≤ 50 ng/ml) also showed a decreasing trend by days of cleanup work (Table 3.9). In the univariable model, the odds ratio (95% CI) of detectable t,t-MA declined to 0.19 (0.07-0.54) on day 15-28 compared to the reference group (day 2-4) with a highly significant trend (P<0.001). The decreasing trends remained significant after adjustment for creatinine and/or cotinine. Among smokers there was no evidence of a decreasing trend in odds ratio of detectable t,t-MA with days of cleanup (data not shown).

Table 3.9: Logistic Regression with GEE* of Detectable t,t-MA by Days of Cleanup (Non-Smokers: Cotinine ≤50 ng/ml) (N=679)

Weeks of Study	Days of Cleanup	Odds Ratio of Detectable t,t-MA (95%CI)			
		Univariable Model	Model 1	Model 2	Model 3
Week 1.1	**Day 2-4**	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Week 1.2	**Day 5-7**	0.74 (0.47-1.17)	0.94 (0.56-1.58)	0.67 (0.41-1.08)	0.84 (0.49-1.44)
Week 2	**Day 8-14**	**0.49 (0.28-0.85)**	0.83 (0.45-1.52)	**0.44 (0.25-0.78)**	0.75 (0.40-1.38)
Week 3-4	**Day 15-28**	**0.19 (0.07-0.54)**	0.34 (0.12-1.01)	**0.17 (0.06-0.44)**	**0.28 (0.10-0.80)**
P-Trend		**<0.001**	0.077	**<0.001**	**0.028**

Model 1: Adjusted by Urinary Creatinine
Model 2: Adjusted by Urinary Cotinine
Model 3: Adjusted by Urinary Creatinine and cotinine
* Generalized Estimating Equation with Exchangeable Correlation Structure
Bold numbers indicate statistically significant results. (P<0.05)

Urinary t,t-MA detectable percentages did not differ substantially among workers by job description (P=0.335 by Fisher's exact test), ranging from 29.4 to 47.8% among job groups with 15 or more workers (Table 3.10). Similarly, by logistic regression, odds of detectable t,t-MA did not differ among non-smoking workers by job description (Table 3.11). Also, there was no evidence for a protective effect of PPE use for benzene exposure, assessed by urinary t,t-MA concentration. The proportion of urine samples with detectable t,t-MA were not different between workers who wore PPEs and those who did not (Appendix Table 3.7, 3.8).

Table 3.10: Urinary t,t-MA Detectable Percentages by Job Description

Job Descriptions	Total (N)	Urinary t,t-MA			
		Detectable		Non-detectable	
		N	Percent	N	Percent
Total	1,343	436	32.5	907	67.5
Oil Dispersant Applicator	17	5	29.4	12	70.6
Contaminated Sand/Trash Removal	778	265	34.1	513	65.9
Environmental Sampling Personnel	9	1	11.1	8	88.9
Oil Vacuum/Oil Slick Removal	315	91	28.9	224	71.1
Supervisor/Health Care Professional	38	10	26.3	28	73.7
Transport Driver/Ship Pilot	23	11	47.8	12	52.2
Support Personnel	61	21	34.4	38	62.3
Others	44	16	36.4	28	63.6
Missing	58	16	27.6	42	72.4

Table 3.11: Logistic Regression with GEE* of Detectable t,t-MA by Job Description (Subgroup Nonsmokers) (N=655)**

Job Descriptions	Odds Ratio of t,t-MA Detectable (95% CI)				
	Univariable Model	Model 1	Model 2	Model 3	Model 4
Support Personnel[***]	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Oil Dispersant Applicator	NA	NA	NA	NA	NA
Contaminated Sand/ Trash Removal	1.05 (0.48-2.29)	1.23 (0.55-2.71)	0.64 (0.28-1.44)	1.13 (0.51-2.51)	0.56 (0.24-1.29)
Environmental Sampling Personnel	NA	NA	NA	NA	NA
Oil Vacuum/ Oil Slick Removal	0.96 (0.42-2.28)	1.03 (0.44-2.39)	0.55 (0.23-1.34)	0.94 (0.40-2.20)	0.49 (0.20-1.19)
Supervisor/ Health Care Professional	0.77 (0.23-2.58)	0.87 (0.26-3.00)	0.49 (0.13-1.82)	0.86 (0.25-2.96)	0.47 (0.12-1.76)
Transport Driver/Ship Pilot	1.38 (0.30-6.27)	1.58 (0.29-8.79)	1.73 (0.31-8.12)	1.33 (0.24-7.23)	1.33 (0.31-5.74)
Others	1.59 (0.56-4.54)	1.40 (0.48-4.05)	0.72 (0.21-2.51)	1.29 (0.45-3.71)	0.65 (0.18-2.29)

Model 1: Adjusted by days of cleanup (day 2-4, day 5-7, day 8-14, day 15-28)
Model 2: Adjusted by days of cleanup and urinary creatinine
Model 3: Adjusted by days of cleanup and urinary cotinine
Model 4: Adjusted by days of cleanup, urinary cotinine and creatinine

NA because 0/6 of the oil dispersant applicator and 0/8 of environmental sampling personnel had detectable t,t-MA levels

* Generalized Estimating Equation with Exchangeable Correlation Structure
** 24 Unknown Job Description
*** Coordinators, PTTGC Corporate Representatives, Visitors, Photographers, and Journalists were grouped as the support personnel.

DISCUSSION

In our study of Rayong oil spill cleanup workers, we examined internal dose of PAHs and benzene to examine factors related to their exposure. The internal dose of PAHs, as measured by urinary 1-OHPG, was highest in individuals who worked during the first 3 days of cleanup work and was significantly lower among individuals who worked in the final week of the study three weeks later. This was consistent with our hypothesis that the exposure levels of PAHs would be the highest in the first week of cleanup and decline thereafter. After adjusting for cotinine and creatinine by regression analysis, the decline in urinary 1-OHPG concentration with days of cleanup remained highly significant. Job descriptions with the highest level of urinary 1-OHPG after adjustment were oil dispersant applicators and contaminated sand/trash handlers. We also observed a decreasing trend by days of cleanup of detectable urinary t,t-MA, a biomarker of benzene exposure. These results demonstrate that oil spill cleanup workers can be exposed to PAH and benzene at concentrations sufficient to be measured internally as metabolites. Furthermore, these exposures occurred after a relatively small spill of only about 50-300 barrels of oil, much less than that of the Deepwater Horizon (5 million barrels)[24] or Hebei (80,000 barrels)[3] oil spills.

Previous studies from the Hebei oil spill measured biomarkers of PAHs in urine, as well as biomarkers of benzene, toluene, ethyl benzene, and xylene (BTEX) (Cheong et al, 2011 and Ha et al, 2012).[9, 10] They reported elevated levels of t,t-MA, mandelic acid (a metabolite of ethylbenzene), and 1-hydroxypyrene in urine samples collected after cleanup, compared to samples collected before participation ($p<0.05$) (Ha et al).[9] Comparing another group of Hebei cleanup workers with an unexposed reference group, they found no difference between the groups in concentrations of biomarkers of PAHs or the four BTEX compounds (Cheong et al).[10] However, they did report a decline in the levels of two PAH biomarkers (1-OHP and 2-naphthol) over the course of several weeks among the cleanup workers. In general, the levels of PAH biomarkers reported in these studies were high overall (1-OHP geometric mean: 0.5 μg/gCr; range: 0.1 - 2.4 μg/gCr, approximately equivalent to ~ 0.69 - 16.5 pmol/ml), even in the unexposed reference group

(GM: 0.6 μg/gCr; range: 0.2 - 1.7 μg/gCr approximately equivalent to 1.38 - 11.70 pmol/ml) compared to other studies.

A number of factors could contribute to differences in exposure between spills and between studies. The half-life of PAHs in crude oil in the environment can range from a few hours up to weeks or months depending on the chemical composition of the oil, the molecular weights of the PAHs, bacterial biodegradation and photolysis.[25, 26] After a spill and during cleanup, low molecular weight (LMW) PAHs would be expected to evaporate within a few days, resulting in the rapid decline in biomarkers, while the higher molecular weight (HMW) PAHs might take a few weeks to evaporate or degrade. Pyrene, the parent compound of 1-OHPG, is of intermediate MW (m=202) having both rapid and slow evaporation characteristics. The Hebei oil spill workers were recruited for study 2 or more weeks after the oil spill occurred[9, 10] thereby reducing expected PAH exposure.

In our study, the median of urinary 1-OHPG among all oil spill workers was 0.79 pmol/ml, with median levels declining from 0.97 pmol/ml when the cleanup began (days 2-4) to 0.32 pmol/ml 4 weeks later (days 22-28). These levels of 1-OHPG are similar to those reported by Kang et al (1995)[27] for steel plant workers (1.82 pmol/ml) and controls (0.38 pmol/ml), in a study that used the same laboratory and method for 1-OHPG analysis as our study. For comparison, the GM of urinary 1-OHPG in non-smokers in the US is 0.16-0.25 pmol/ml[28], and 0.025 μmol/molCr (approximately equivalent to ~0.38 pmol/ml) in rural non-smokers in Thailand.[29] Thus, the 1-OHPG levels we observed were comparable to occupational exposures during the early days of cleanup, and declined to near background (general population) levels by the end of the cleanup operations (0.18 pmol/ml in non-smokers).

We also examined the levels of urinary 1-OHPG among cleanup workers with different job descriptions. We found that certain types of jobs including, oil dispersant applicators, contaminated sand/trash removal workers and oil vacuum/oil slick removal workers, had higher levels of urinary 1-OHPG than other workers and support personnel. Oil dispersant applicators might be at increased risk of PAH exposure because spraying dispersants on oil-water interfaces generates aerosols that

are respirable (Ehrenhauser et al, 2013). Water wave action on the sea while applying dispersants can also facilitate aerosolization and evaporation of PAHs (Ehrenhauser et al, 2013). Workers dealing with contaminated sand/trash removal and oil vacuum/oil slick removal were often in close (or direct) contact with crude oil, thereby enhancing the possibility of dermal contamination. Thus, these workers might be expected to have higher levels of exposure than other workers or support personnel who did not directly contact crude oil. The study of Ha et al. (2012) among the Hebei oil spill cleanup workers explored the association between PAH metabolites and job types, but did not find any differences in PAH metabolite levels between "direct cleanup jobs" and "logistics-related jobs"[9], the only categories reported.

We also examined the potential effect of PPE use on PAH exposure among cleanup workers. Unexpectedly, levels of 1-OHPG were not associated with overall PPE use, consistent with the finding of Lee et al. (2009) from Hebei oil spill.[30] Furthermore, individual equipment use (masks, gloves, boots, or coveralls) was not associated with a protective effect. This suggests that PPE was not effective, or was not used properly, or that the questionnaire data was not reliable. Paradoxically, mask and coverall use were associated with elevated levels of 1-OHPG. This might have resulted from exposure selection bias because of higher hazard recognition (resulting in enhanced PPE use) in the early days of cleanup when the beach was covered in oil, compared to later weeks of cleanup. About 60% of workers who worked in the first 3 days of cleanup (when exposure was high) wore masks or coveralls, whereas only 1-10% of workers during the last 2 weeks of cleanup wore them. In addition to exposure recognition, masks can be contaminated accidentally by direct contact with oil soaked gloves. Another complicating factor is the possible limitation of supply of PPE which would be expected to restrict PPE use. For example, boot and glove use increased over the course of cleanup from 37% (boots) and 47% (gloves) during the first 3 days of cleanup, to 62% and 78%, respectively, during the last 2 weeks of cleanup.

We found that the percentage of oil spill workers with detectable urinary t,t-MA decreased from day 2-4 to week 3-4 of cleanup in nonsmokers, but not in smokers. The method used to

measure t,t-MA in these workers[20] had limited sensitivity, with an estimated limit of quantification of 0.10 μg/ml. This compares unfavorably with the background t,t-MA level in the general population of 0.07 μg/ml (range: 0.02-0.30 μg/ml).[31, 32] Because of the high percentage (67.5%) of samples that were not detectable in our study, we analyzed the t,t-MA data as a binary variable. This limited our statistical power to detect associations[33] and might partially explain why we did not observe a decreasing trend with time of cleanup in smokers. In addition, tobacco smoke contains benzene and significantly increases concentration of urinary t,t-MA in smokers compared to nonsmokers.[34] Although we did adjust for smoking by including urinary cotinine concentration in regression models, this adjustment may not have been sufficient to completely control for the confounding effects of benzene from smoking. In addition, sorbic acid-containing foods can artificially increase levels of urinary t,t-MA, apart from exposure to benzene, and this was not controlled for in our analysis. Compared to 1-OHPG, the percentage of detectable t,t-MA samples among nonsmokers decreased more rapidly with days of cleanup than the levels of 1-OHPG. The rapid decline in detectable t,t-MA, is not unexpected as benzene is relatively more volatile than PAHs, and would be expected to evaporate within a few days of the oil spill. Detectable urinary t,t-MA was not found to be related to job descriptions as was urinary 1-OHPG. This lack of association could be due to the rapid evaporation of benzene or the lack of statistical power. In addition, urinary t,t-MA was not associated with PPE use, similar to our findings on PPE use and urinary 1-OHPG.

Urinary t,t-MA measured in other studies of oil spill workers is somewhat limited. Ha et al (2012) found that levels of urinary t,t-MA of workers at the Hebei spill were higher after cleanup participation compared to levels before participation among both smokers (2.5-fold higher) and nonsmokers (3.2-fold higher).[9] In contrast, among another group of Hebei spill cleanup workers, Cheong et al (2011) found no difference in t,t-MA levels between workers and unexposed controls, and no change in t,t-MA levels between weeks 2-3 and weeks 5-6 of cleanup.

Our study is the first investigation of PAH and benzene biomarkers in cleanup workers' urine samples that were collected within two days of a fresh oil spill. In contrast to the studies of cleanup workers at the most intensively investigated spill, the Hebei oil spill, where urine sample collection started 2 weeks after cleanup started, our study assessed internal dose of PAHs and benzene beginning on the 2nd day of cleanup, at which time exposure was expected to be close to maximum. To our knowledge, none of the studies of oil spill incidents that incorporated exposure biomarkers had access to urine samples collected on the first few days of cleanup. In addition, our study had a relatively large sample size (n=1343) compared to the three studies from the Hebei oil spill (n=121, n=154, n=724)[9,10,32] and the study of D'Andrea and Reddy (2014)[35] from the Deepwater Horizon oil spill (n=117). In the current study, we also used urinary cotinine to adjust for expected confounding effects of smoking on PAH biomarkers.

The current analysis has several limitations. It employs a cross-sectional exposure analysis, thus limiting our ability to assess causal inference. Secondly, the questionnaire data was initially designed as part of a health surveillance program initiated by the Rayong Provincial Health office, rather than a formal scientific study. As a result, some of the data, such as hours of cleanup participation, smoking status, dietary patterns, and pre-exposure assessment was not complete or unavailable for statistical analysis. Third, we were unable to assess possible confounding effects due to diet, including sorbic acid-containing foods affecting t,t-MA[36] and PAH-containing foods, such as broiled and smoked meats, affecting 1-OHPG,[37, 38] that may have resulted in either underestimating or overestimating our results. Urinary S-phenyl mercapturic acid, which is more specific for benzene than t,t-MA, might be a better biomarker to use in future studies. Fourth, our study did not have an ideal negative control population that was absolutely unexposed to crude oil, such as pre-cleanup baseline measurements of workers, or non-participants who were not involved in the cleanup. For these reasons, it is difficult to assess the magnitude of the increase in levels of PAH and benzene biomarkers among the oil spill cleanup workers on the first days of the spill. In addition, genetic polymorphisms in Phase I enzymes, such as CYP1A1 and CYP1B1[39], and Phase

II enzymes, such as glutathione S-transferases (GSTs), N-acetyltransferase-1 (NAT1) and epoxide hydrolase (EPHX1), might explain some of the variation in the levels of urinary 1-OHPG and t,t-MA that we observed.

This study will serve as the baseline exposure assessment and characteristics of workers for future research from the Rayong oil spill cohort. The health followup of these workers at Rayong Hospital is ongoing and planned to last 5 years. Since our study found evidence of moderate to high exposure to carcinogenic substances, PAHs and benzene, we believe that long term surveillance of these workers is prudent.

In conclusion, Rayong oil spill cleanup workers exhibited evidence of elevated levels of PAH and benzene exposure during the early days of cleanup, compared to near background levels 4 weeks after cleanup began. Certain types of jobs including, oil dispersant applicators, contaminated sand/trash removal workers, and oil vacuum/oil slick removal workers, were at highest risk of PAH exposure. Long-term health monitoring of oil spill cleanup workers should be implemented.

Acknowledgement

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CHAPTER 4: POST-SHIFT SYMPTOMS AMONG RAYONG OIL SPILL CLEANUP WORKERS

ABSTRACT

Background: In July of 2013, a pipeline connecting an offshore oil platform to a tanker caused crude oil to spill into the Sea of Rayong off the coast of Thailand. The estimated amount of oil spilled was between 50 and 190 cubic meters or 336-1,200 barrels. The resulting oil slick washed ashore one day later on the island of Samet. On-land cleanup lasted about a month and was performed by a combination of territorial defense volunteers, citizen volunteers, Thai military personnel and company employees. We examined prevalence of post-shift acute symptoms among Rayong oil spill cleanup workers, and assessed their association with predictive factors, including internal dose biomarkers of PAH and benzene exposure, day of cleanup worked, job description, PPE use, and age of workers.

Methods: Post-shift acute symptoms and other variables recorded by questionnaire were linked to internal dose estimates based on urinary concentrations of 1-hydroxypyrene-glucuronide (1-OHPG), trans,trans-muconic acid (t,t-MA), and cotinine. Logistic regression models were used to assess the association between each of 23 physical symptoms and urinary 1-OHPG concentration or t,t-MA detectable levels, adjusting for day/week of cleanup worked, and urinary cotinine. Cleanup job descriptions, personal protective equipment use, and age of workers, were also examined as potential modifying factors. Symptom groups were also determined by exploratory factor analysis. The association between 7 symptom groups and potential exposure variables was then assessed by ordinal logistic regression models.

Results: Prevalence of several post-shift symptoms, including irritation of throat and nose, increased with concentration of urinary 1-OHPG, an internal dose biomarker of PAHs, with an odds ratio (95% CI) of 1.04 (1.00 - 1.08) per 1 pmol/ml increase in 1-OHPG. Similarly, one group of symptoms determined by factor analysis, designated as “irritative symptoms”, including irritation of the eye, throat and/or nose, eye injection (redness) and excessive tearing (epiphora)

was associated with increased concentration of urinary 1-OHPG (OR = 1.03; 95% CI = 1.00-1.06, per 1 pmol/ml increase in 1-OHPG). After adjusting for cotinine and day of cleanup worked, this association remained significant. Unexpectedly, the prevalence of reported symptoms was higher in support personnel than in workers performing cleanup jobs with more direct potential exposure to oil. None of the symptoms were related to urinary t,t-MA concentration, a biomarker of benzene exposure, or PPE use.

Conclusion: Rayong oil spill cleanup workers exhibited evidence of an association between prevalence of acute irritative symptoms and PAH exposure measured by urinary 1-OHPG. Long-term health monitoring of oil spill cleanup workers should be implemented, particularly among those workers suspected of sustaining high exposure to crude oil.

INTRODUCTION

On 27 July, 2013, a pipeline connecting an offshore oil platform to a tanker, operated by PTT Global Chemical (PTTGC), a corporation owned by the government of Thailand, leaked and caused crude oil to spill into the Sea of Rayong off the coast of Thailand.[1] The crude oil covered an area of approximately 20 square kilometers and washed ashore on the island of Samet in an area called "Ao Prao" on 28 July, 2013.[2] The estimated amount of oil spilled was between 50 and 190 cubic meters or 336-1,200 barrels.[1] On-land cleanup lasted about a month and was performed by a combination of territorial defense volunteers, citizen volunteers, Thai military personnel and PTTGC employees. Cleanup procedures included oil containment, skimming, and dispersal, using absorbent pads, high-pressure water spraying and removal and disposal of contaminated soil, sand and rocks.[2]

A recent study quantified internal dose of polycyclic aromatic hydrocarbons (PAHs) and benzene in these workers to examine their potential exposure. The internal dose of PAHs as measured by urinary 1-hydroxypyrene-glucuronide (1-OHPG) was highest in individuals who worked during the first 3 days of cleanup work (median: 0.97 pmol/ml) and was 66.7% lower (median: 0.32 pmol/ml) among individuals who worked in the final week of the study (days 21-28). This suggests that the exposure levels of PAHs were highest in the first week of cleanup and declined thereafter (Chapter 3). The percentage of cleanup worker's urine samples with detectable levels of t,t-muconic acid (t,t-MA), a metabolite of benzene, also declined over the four-week cleanup period (limit of quantitation of t,t-MA ~0.10 ug/ml).

The International Agency for Research on Cancer (IARC) has classified benzene and some PAHS, including benzo[a]pyrene, as group 1 carcinogens, known to cause cancer in humans[3]. PAHs from occupational exposure are associated with respiratory and urinary tract cancers.[4] Workers exposed to PAHs report various symptoms, including breathing problems, chest pains, chest irritation, throat irritation and cough.[5] PAHs are also associated with asthma and allergy in children.[6] Sub-chronic to chronic exposure to benzene can result in pancytopenia, aplastic anemia

and eventually leukemia -- especially acute myelogenous leukemia (AML).[7-14] Whereas acute inhalation of high doses of benzene (> 60 ppm) can result in neurotoxic symptoms, including dizziness, headache and drowsiness.[15]

In the current study, we examined prevalence of post-shift acute symptoms among Rayong oil spill cleanup workers, and assessed their association with predictive factors, including internal dose biomarkers of PAH and benzene exposure, day of cleanup worked, job description, PPE use, and age of workers.

MATERIALS AND METHODS

The symptom and related data were first collected as part of the health surveillance for oil spill cleanup workers. The consent for use of this data for scientific study was obtained by the Rayong Hospital and the Thai Naval Medical Department. Approval for the further analysis of de-identified data in our study was approved by the institutional review board of the Johns Hopkins Bloomberg School of Public Health, and the ethical committees of the Prince of Songkla University, Rayong Hospital, and the Thai Naval Medical Department.

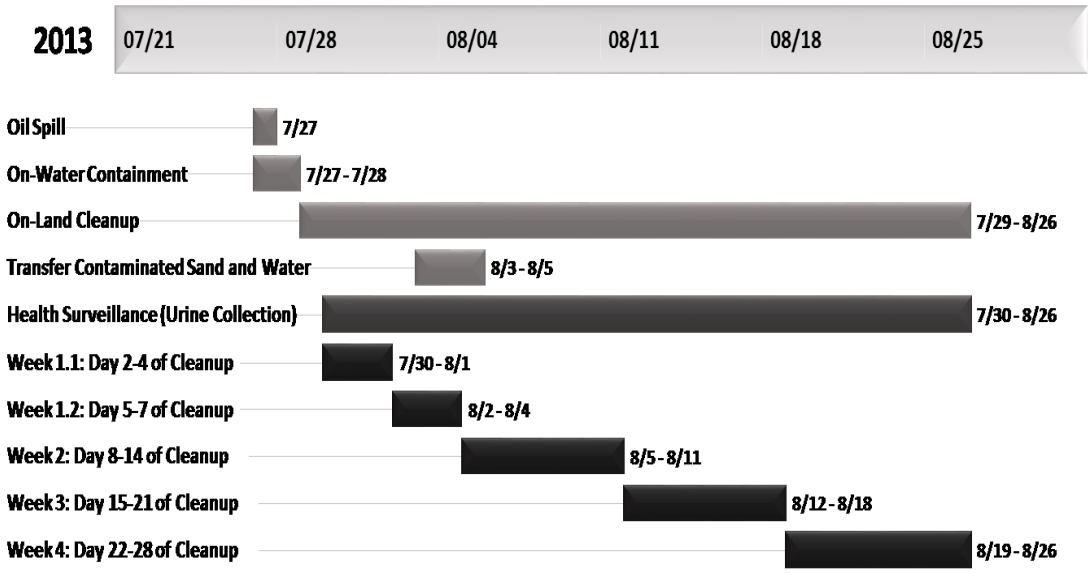
Study Population and Questionnaire

The study population consisted of workers who participated in the Rayong oil spill cleanup of July-August, 2013. It included workers from a variety of backgrounds (territorial defense volunteers, citizen volunteers, Thai military personnel and PTTGC employees) who were recruited into a health surveillance program by Rayong Hospital and the Thai Naval Medical Department. Our study used data from the Rayong Hospital symptoms questionnaire and our internal dose measurements of urinary PAH and benzene metabolites from our exposure study of these workers (Chapter 3). The questionnaire was designed by a team composed of committees from Rayong Provincial Public Health Office, Rayong Hospital, Thai Naval Medical Department and Thai Department of Disease Control. Workers who provided urine were asked to answer self-reported questions regarding their post-shift acute physical symptoms at the time their urine was collected. The data from the questionnaire was linked to the 1,343 usable urine samples that we had analyzed previously for 1-OHPG and t,t-MA.

Details of the Rayong Oil Spill Cleanup Study

The date that the on-land cleanup began (29 July 2013) was counted as Day 1 of cleanup in our study. The health surveillance protocol, including questionnaire and urine sample collection, began the next day (Day 2). A calendar depicting the cleanup sequence and our study time periods is shown below (Figure 4.1).

Figure 4.1: Rayong Oil Spill Cleanup Study (27 July 2013 – 26 August 2013)



Demographic factors and their distribution are shown previously in Table 3.1 in Chapter 3. Of 1,343 oil spill study cleanup workers, 93.2% were males. The median age was 27 years old (Interquartile Range (IQR) = 18.0) and the majority (55.3%) of the workers' background occupations were military personnel. Forty percent of the workers participated in the oil spill cleanup on Day 2 to Day 4 of the cleanup, and 24.4% of the workers participated on Day 5 to Day 7 of cleanup. Working hours per day was not available in the questionnaires. The workers performed various oil spill cleanup jobs. Of 1,343 workers, 57.9% were assigned the job to manually remove oil-contaminated sand, rocks, and trash (Table 3.2), and 23.5% were assigned the job to vacuum or manually remove the oil slick from water.

Workers were also asked about their personal protective equipment (PPE) use. They were asked if they wore any PPE, an N95 mask, an R95 mask, any mask with filter, coveralls, gloves or boots. The mask questions were grouped as "any mask use" if the workers answered "yes" to at least one of the questions, regarding the use of N95, R95 or mask with filter. Most of the workers (84%) self-reported using at least one piece of PPE (either mask, coveralls, gloves or boots) during

their shifts (Table 3.3). However, only 16.8% of the workers wore the complete set of PPE, and 31.7% reported that they **"**often**"** wore at least one piece of PPE.

Laboratory Methods

Urinary 1-hydroxypyrene-glucuronide (1-OHPG), a measurable metabolite of pyrene in urine, was measured to assess the internal dose of PAH of each worker. Urinary 1-OHPG was measured by immunoaffinity chromatography and synchronous fluorescence spectroscopy (SFS), modified from protocols of Strickland et al. (1994)[16] as described in our exposure assessment study (Chapter 3). The limit of detection (LOD) was 0.04 pmol/ml, the recovery of the assay was 82% and the coefficient of variation was 5.6%.

Urinary t,t-MA levels, measured by HPLC with fluorescence detection, were retrieved from Rayong Hospital data.[17] The coefficient of variation was 71-85% and the recovery rate was 91-95%.[17] The limit of quantitation (LOQ) was estimated to be 0.01 mg/dl or 0.10 µg/ml.

Creatinine and cotinine were measured as previously described (Chapter 3). The coefficient of variation of creatinine was 5% and the limit of detection was 0.1 mg/dl. The coefficient of variation of cotinine was 8% and the limit of detection was 2 ng/ml. We used the generally recommended cut-off of 50 ng/ml of urinary cotinine to differentiate between non-smokers and passive smokers.[18, 19]

Statistical Methods

All available questionnaire data was linked to the 1-OHPG, t,t-MA, and cotinine measurements. Non-detectable measurements of urinary 1-OHPG and cotinine were replaced with the value of the LOD/$\sqrt{2}$. The prevalence of symptoms by categorical variables, such as day of cleanup, PPE use, smoking status (cotinine concentration), age of workers, or job description was explored. Because of the large proportion of non-detectable samples (67.5%), the urinary t,t-MA data was analyzed as a binary variable (detectable vs non-detectable). In addition, due to the relatively smaller sample size, t,t-MA data from the 3rd and 4th weeks of cleanup were combined before the statistical analysis.

For inferential statistics, logistic regression models were used to assess the association between each physical symptom and urinary 1-OHPG concentration or t,t-MA detectable levels, adjusting for days of cleanup (day 2-4, day 5-7, week 2, week 3-4 of cleanup) and urinary cotinine.

A latent variable approach, exploratory factor analysis (EFA), was used to assess the underlying correlation between the post-shift symptoms. This method uses the correlation patterns among variables to group them into underlying latent factors, without a priori hypothesis of the measured factors and patterns. Based on Bayesian information criterion and the VSS (very simple structure) model selection method described by Revelle and Rocklin (1979)[20], the optimal number of symptom factors selected was 7. The root mean square error of approximation (RMSEA) index of the EFA 7 factor model was 0.05, indicating good model fit.[21] To explore the factor (group) structures of the post-shift symptoms, the weighted least squares factor analysis described by Harman et al (1966)[22] was used. The direct oblimin rotation procedure was used to allow correlations between factors using a correlation cut-off of 0.3. Each symptom in the 7 groups was assigned a score of 1, with each symptom group having a cumulative score ranging up to 5 depending on the number of symptoms in the group (see factor analysis results). Ordinal logistic regression models were used to assess the association between the 7 symptom groups and potential exposure variables.

Sensitivity analysis by adding age of workers as an adjusted variable in the logistic regression models of symptoms and 1-OHPG and t,t-MA was performed. The results were not different from the models without age. Therefore, we report results from the models without age of workers (See Appendix Table 4.4 and 4.7).

All statistical analysis was completed using R version 3.2.4. (R Development Core Team, Vienna, Austria, 2016).

RESULTS

Prevalence of Post-Shift Symptoms

Acute physical symptoms were assessed by questionnaire completed by workers immediately after their work shift. The questionnaire consisted of 23 items regarding post-shift symptoms, including dizziness, irritated throat, irritated eye, muscle pain, irritated nose, sore throat, cough, heavy breathing, running nose, skin itching, nausea, blurred vision, feeling faint, abdominal pain, excessive tearing of eyes (epiphora), diarrhea, eye injection (redness), eczema, chest tightness, palpitation, injuries, taste change and vomiting. Overall, 36.3% of workers reported having at least one of the symptoms. The 5 symptoms with the greatest prevalence were dizziness (10.6%), irritated throat (9.2%), irritated eye (8.9%), muscle pain (7.8%) and irritated nose (7.4%) (Table 4.1).

Self-reported dizziness was the only symptom that showed a decreasing trend in prevalence as cleanup progressed through the full 4 weeks (P-trend=0.005) (Table 4.2). Other symptoms including irritated throat, irritated eyes, muscle pain, sore throat, cough, running nose, skin itching, abdominal pain, diarrhea and injuries showed increasing trends over the 4 weeks of cleanup (P-value <0.05). Some of these symptoms (cough, runny nose and abdominal pain) may have been due to stomach flu and were concentrated in the military personnel (Appendix Table 4.12).

Table 4.1: Prevalence of 23 self-reported symptoms

Post-shift Symptoms	Prevalence (n)	%
Total Number	1343	100.0
Missing Symptoms Data	25	1.9
Any Symptoms	487	36.3
Dizziness	142	10.6
Irritated Throat	124	9.2
Irritated Eyes	120	8.9
Muscle Pain	105	7.8
Irritated Nose	99	7.4
Sore Throat	80	6.0
Cough	75	5.6
Heavy Breathing	70	5.2
Running Nose	68	5.1
Skin Itching	39	2.9
Nausea	38	2.8
Blurred Vision	31	2.3
Feeling Faint	27	2.0
Abdominal Pain	25	1.9
Excessive Tearing of Eyes (Epiphora)	24	1.8
Diarrhea	21	1.6
Eye Injection (Redness)	20	1.5
Eczema	19	1.4
Chest Tightness	16	1.2
Palpitation	16	1.2
Injuries	13	1.0
Taste Change	8	0.6
Vomiting	3	0.2

Table 4.2: Prevalence of Symptoms by Days of Cleanup

Post-Shift Symptoms	Day 4-7	Day 5-7	Week 2	Week 3-4	P-Trend*
	% Prevalence	% Prevalence	% Prevalence	% Prevalence	
Total Number	**537**	**328**	**282**	**196**	
Dizziness	12.1	13.7	6.4	7.1	**0.005** ↓
Irritated Throat	6.1	13.1	8.5	12.2	**0.027** ↑
Irritated Eyes	8.9	13.7	5.3	6.1	0.053
Muscle Pain	3.7	8.2	10.3	14.8	**<0.001** ↑
Irritated Nose	8.8	6.7	6.0	6.6	0.157
Sore Throat	2.0	6.4	7.4	13.8	**<0.001** ↑
Cough	1.1	3.7	4.3	23.0	**<0.001** ↑
Heavy Breathing	5.4	6.1	2.8	6.6	0.770
Running Nose	3.0	1.8	3.9	17.9	**<0.001** ↑
Skin Itching	1.3	2.7	5.7	3.6	**0.005** ↑
Nausea	2.6	4.0	2.8	1.5	0.533
Blurred Vision	1.5	3.7	2.8	1.5	0.660
Feeling Faint	1.7	2.1	0.4	5.1	0.106
Abdominal Pain	0.4	1.5	2.5	5.6	**<0.001** ↑
Eye Tearing (Epiphora)	0.9	2.7	1.4	3.1	0.113
Diarrhea	0.2	0.9	2.5	5.1	**<0.001** ↑
Eye Injection (Redness)	0.7	2.7	1.4	1.5	0.428
Eczema	0.6	0.9	3.9	1.0	**0.035** ↑
Chest Tightness	0.6	2.1	1.8	0.5	0.601
Palpitation	0.7	2.7	1.1	0.0	0.528
Injuries	0.4	0.6	1.8	2.0	**0.014** ↑
Taste Change	0.4	1.2	0.4	0.5	0.966
Vomiting	0.2	0.0	0.4	0.5	0.375

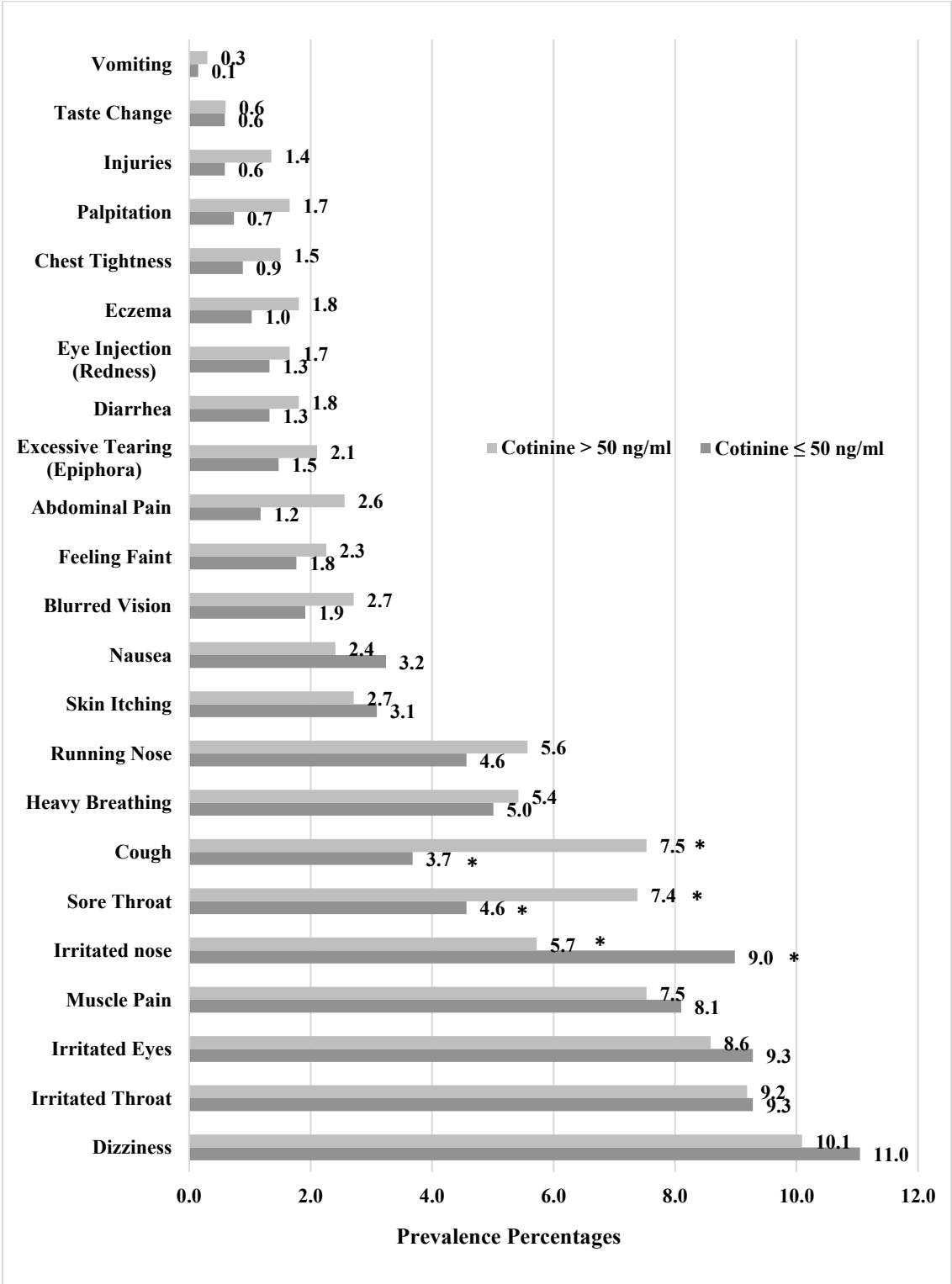
*P-trend was calculated by Mantel-Haenstzel chi-squared test for trend. ↓ Decreasing trend; ↑ Increasing trend.

Bold numbers indicate statistically significant results. (P<0.05)

Post-Shift Symptoms and Urinary Cotinine

Certain symptoms, including, irritated nose, cough and sore throat were associated with smoking, classified as urinary cotinine more than 50 ng/ml (Appendix Table 4.1 and Figure 4.2). Prevalence of sore throat and cough (7.4% and 7.5%, respectively) in the smokers (cotinine > 50 ng/ml) was greater than the prevalence in nonsmokers (cotinine $\leq$ 50 ng/ml) (4.6% and 3.7%, respectively). The prevalence of irritated nose was lower in smokers (5.7%) than in nonsmokers (9.0%). These findings suggested that smoking might confound the association between exposure variables (e.g., 1-OHPG, t,t-MA) and physical symptoms. Therefore, cotinine was used as an adjustment variable when assessing associations between symptoms and 1-OHPG or t,t-MA.

Figure 4.2: Prevalence of Post-Shift Symptoms by Cotinine Levels (Less than or greater than 50 ng/ml)



***Indicating significant differences (P<0.05)**

Post-Shift Symptoms and Age of Workers

Age of workers was also related to prevalence of symptoms. Workers aged 40 years or older reported higher prevalence of several post-shift symptoms, including irritated throat, eyes and nose, blurred vision and eye injection (redness), compared to worker younger than 40 years of age. Whereas, cough, runny nose and abdominal pain were reported less frequently in workers 40 years or older, compared to younger workers (Table 4.3). These latter three symptoms (cough, runny nose and abdominal pain) may have been due to stomach flu and were concentrated in the military personnel, who were relatively younger (Appendix Table 4.2).

Table 4.3: Prevalence of Post-Shift Symptoms Stratified by Age (N=1,311)*

Post-Shift Symptoms	Total	Age < 40 yrs		Age >= 40 yrs		P-value**
	N	N	%	N	%	
Total Number	1,311	982	100.0	329	100.0	
Dizziness	141	105	10.7	36	10.9	0.899
Irritated Throat	123	80	8.1	43	13.1	**0.011**
Irritated Eyes	120	80	8.1	40	12.2	**0.029**
Muscle Pain	105	77	7.8	28	8.5	0.699
Irritated Nose	99	57	5.8	42	12.8	**<0.001**
Sore Throat	80	62	6.3	18	5.5	0.581
Cough	75	69	7.0	6	1.8	**<0.001**
Heavy Breathing	70	50	5.1	20	6.1	0.491
Running Nose	68	58	5.9	10	3.0	**0.042**
Skin Itching	39	26	2.6	13	4.0	0.228
Nausea	37	24	2.4	13	4.0	0.153
Blurred Vision	31	18	1.8	13	4.0	**0.029**
Feeling Faint	27	25	2.5	2	0.6	0.032
Abdominal Pain	25	23	2.3	2	0.6	**0.046**
Eye Tearing (Epiphora)	24	15	1.5	9	2.7	0.157
Diarrhea	21	17	1.7	4	1.2	0.519
Eye Injection (Redness)	20	10	1.0	10	3.0	**0.010**
Eczema	19	15	1.5	4	1.2	0.682
Chest Tightness	16	11	1.1	5	1.5	0.568
Palpitation	16	12	1.2	4	1.2	0.993
Injuries	13	12	1.2	1	0.3	0.204
Taste Change	8	6	0.6	2	0.6	1.000
Vomiting	3	2	0.2	1	0.3	1.000

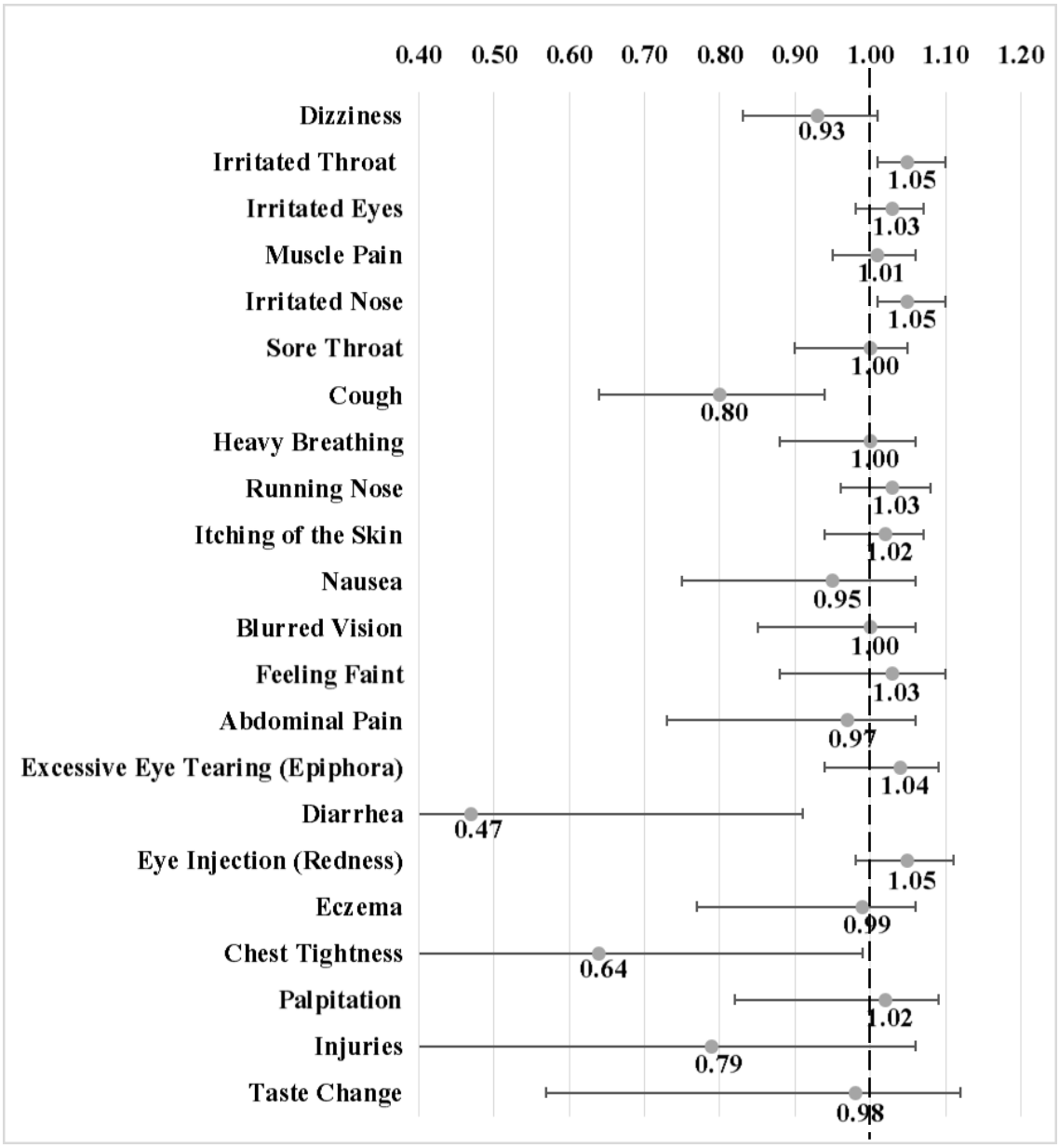
***32 missing data (either symptoms or age)**

****P-value from Fisher's exact test or chi-squared test**

Logistic Regression of Post-shift Symptoms and Internal Dose (1-OHPG and t,t-MA)

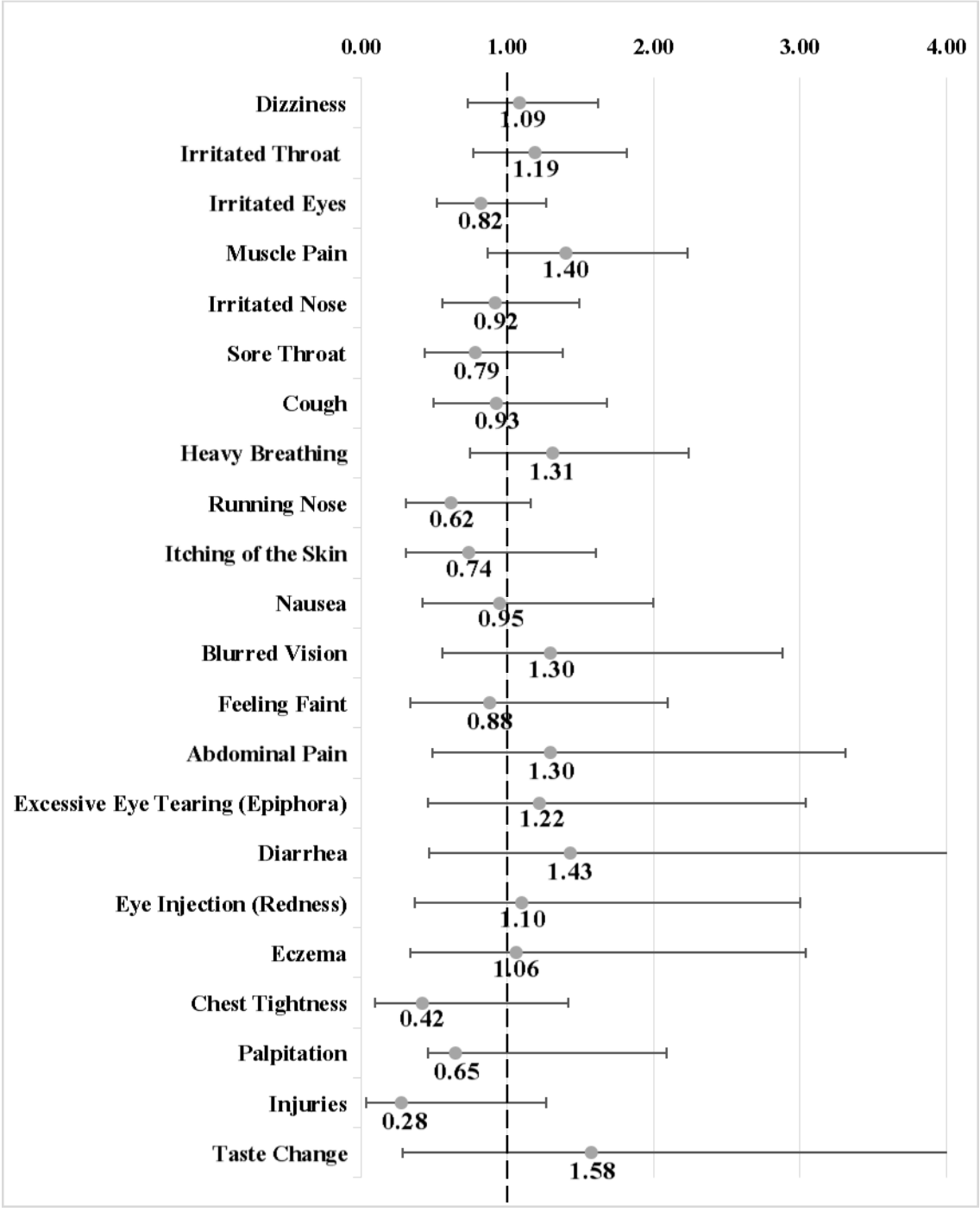
Post-shift symptoms that were significantly associated with urinary 1-OHPG levels included irritated throat, irritated nose, muscle pain, irritated nose, cough, diarrhea and chest-tightness (Figure 4.3). The risk (95% CI) of having irritated throat or irritated nose increased 1.05 (1.01-1.10) and 1.05 (1.01-1.10) fold respectively, per 1 pmol/ml increase in 1-OHPG. Whereas, the risk (95% CI) of having cough, diarrhea or chest tightness was 0.80 (0.64-0.94), 0.47 (0.18-0.91) and 0.64 (0.33-0.99) fold respectively, per 1 pmol/ml increase in 1-OHPG (Appendix Table 4.3). Although urinary 1-OHPG appeared to be protective for these latter 3 symptoms, they may have been considered serious enough to cause these workers to stop working early, thereby reducing their exposure levels. Although not significant, other irritation symptoms, including irritated eye, eye injection (redness) and tearing (epiphora) were also positively associated with urinary 1-OHPG (Figure 4.3, Appendix Table 4.3 - 4.4). None of the post-shift symptoms were associated with detectable levels of urinary t,t-MA except irritated throat (Figure 4.4, Appendix Table 4.5 - 4.6).

Figure 4.3: Adjusted Odds Ratio* of Post-Shift Symptoms (per 1 pmol/ml change in 1-OHPG)



*** Odds ratio adjusted by urinary cotinine and days of cleanup (Multivariable Model)**

Figure 4.4: Adjusted Odds Ratio* of Post-Shift Symptoms by Detectable t,t-MA



*** Odds ratio adjusted by urinary cotinine and days of cleanup (Multivariable Model)**

Post-Shift Symptoms and Job Description

Due to the small number of workers in most job categories with physical symptoms, we were only able to examine the distribution of post-shift symptoms in two high PAH exposure job descriptions (contaminated sand/trash removal and oil vacuum/oil slick removal) and one relatively low PAH exposure job description (support personnel). For this reason, the total number of workers in this section of the analysis was 1,150 (rather than 1,343) (Table 4.4). Prevalences of irritated throat, irritated eyes, irritated nose, and heavy breathing, were higher in support personnel than in workers involved in removing contaminated sand/trash or vacuuming/removing oil slicks ($P<0.05$ from chi-squared or fisher's exact test). Whereas, prevalence of cough was highest in the workers whose job was to vacuum/remove oil slicks ($P=0.025$). Because the support personnel group tended to be older than the other two groups, they may have had more frequent background symptoms (prior to involvement with the cleanup), or be more sensitive to heat-related symptoms or toxicants present during the cleanup work (Appendix Table 4.8). None of the support personnel reported having cough, feeling faint, chest tightness or palpitation symptoms. In our regression analysis, we chose the support personnel as the reference group because they had the lowest median levels of urinary 1-OHPG from our exposure assessment study (Chapter 3). Adjusting for days of cleanup and urinary cotinine, the odds of having irritation of throat and nose, sore throat, heavy breathing and nauseate in contaminated sand/trash removal workers was significantly lower than in support personnel (Table 4.5). Whereas, in oil vacuum/oil slick removal workers, only the odds of having irritation of throat and nose, and heavy breathing was significantly lower than in support personnel (Table 4.5). Results from the univariable model and the model further adjusted for age of workers are shown in Appendix Table 4.9 and Appendix Table 4.10.

Table 4.4: Prevalence of Post-Shift Symptoms by Job Description (n=1,150)

Post-Shift Symptoms	Total	Support* Personnel		Contaminated Sand/Trash Removal		Oil Vacuum/ Oil Slick Removal		P-value**
	N	N	%	N	%	N	%	
Total Number	1,150	60	100.0	775	100.0	315	100.0	
Dizziness	123	8	13.3	83	10.7	32	10.2	0.713
Irritated Throat	99	15	25.0	64	8.3	20	6.3	**<0.001**
Irritated Eyes	97	7	11.7	54	7.0	36	11.4	**0.039**
Muscle Pain	93	3	5.0	64	8.3	26	8.3	0.754
Irritated Nose	85	11	18.3	51	6.6	23	7.3	**0.009**
Sore Throat	74	7	11.7	46	5.9	21	6.7	0.205
Cough	73	0	0.0	58	7.5	15	4.8	**0.019**
Heavy Breathing	60	8	13.3	39	5.0	13	4.1	**0.025**
Running Nose	65	2	3.3	51	6.6	12	3.8	0.172
Skin Itching	32	3	5.0	17	2.2	12	3.8	0.135
Nausea	34	6	10.0	18	2.3	10	3.2	**0.010**
Blurred Vision	23	2	3.3	12	1.5	9	2.9	0.182
Feeling Faint	27	0	0.0	16	2.1	11	3.5	0.192
Abdominal Pain	23	1	1.7	16	2.1	6	1.9	1.000
Eye Tearing	19	1	1.7	13	1.7	5	1.6	1.000
Diarrhea	20	1	1.7	14	1.8	5	1.6	1.000
Eye Injection	11	2	3.3	7	0.9	2	0.6	0.155
Eczema	16	1	1.7	6	0.8	9	2.9	**0.028**
Chest Tightness	14	0	0.0	11	1.4	3	1.0	0.891
Palpitation	14	0	0.0	11	1.4	3	1.0	0.891
Injuries	12	1	1.7	5	0.6	6	1.9	0.108
Taste Change	7	1	1.7	4	0.5	2	0.6	0.453
Vomiting	2	0	0.0	2	0.3	0	0.0	1.000

*Coordinators, PTTGC Corporate Representatives, Visitors, Photographers, and Journalists were grouped as support personnel.

****P-value from Chi-squared or Fisher's exact test**

Bold numbers indicate statistically significant results. (P<0.05)

Table 4.5: Adjusted Odds Ratio of Post-Shift Symptoms by Job descriptions (n=1,150)

Post-shift Symptoms	Support Personnel*	Contaminated Sand/ Trash Removal		Oil Vacuum/ Oil Slick Removal	
	OR	OR**	(95% CI)	OR**	(95% CI)
Dizziness	1.00 (Ref)	1.02	(0.48-2.41)	0.94	(0.42-2.33)
Irritated Throat	1.00 (Ref)	**0.25**	**(0.13-0.50)**	**0.21**	**(0.10-0.45)**
Irritated Eyes	1.00 (Ref)	0.74	(0.33-1.88)	1.28	(0.56-3.37)
Muscle Pain	1.00 (Ref)	1.60	(0.55-6.81)	1.91	(0.62-8.38)
Irritated Nose	1.00 (Ref)	**0.32**	**(0.16-0.70)**	**0.34**	**(0.15-0.78)**
Sore Throat	1.00 (Ref)	**0.34**	**(0.14-0.91)**	0.51	(0.20-1.42)
Cough	1.00 (Ref)	**NA**			
Heavy Breathing	1.00 (Ref)	**0.37**	**(0.16-0.91)**	**0.32**	**(0.12-0.90)**
Running Nose	1.00 (Ref)	1.05	(0.29-6.74)	0.84	(0.21-5.65)
Skin Itching	1.00 (Ref)	0.34	(0.10-1.54)	0.59	(0.17-2.76)
Nausea	1.00 (Ref)	**0.31**	**(0.12-0.91)**	0.39	(0.13-1.23)
Blurred Vision	1.00 (Ref)	0.59	(0.15-3.94)	1.11	(0.26-7.66)
Feeling Faint	1.00 (Ref)	**NA**			

*Coordinators, PTTGC Corporate Representatives, Visitors, Photographers, and Journalists were grouped as support personnel.

****Odds ratio adjusted by urinary cotinine and days of cleanup (Multivariable Model)**

Bold numbers indicate statistically significant results. ($P<0.05$)

NA because the prevalence of symptoms in the reference group is too small to calculate the odds ratio: 0/60 in cough, 0/60 feeling faint

For abdominal pain, epiphora, diarrhea, eye injection, eczema, chest tightness, palpitation, injuries, taste change and vomiting, the number of cases is too small to calculate accurate odds ratio.

Post-shift Symptoms and Personal Protective Equipment (PPE) Use

Using at least one piece of PPE was associated with decreased prevalence of cough, diarrhea, and chest tightness, but increased prevalence of irritated throat and nose (Table 4.6). Using a mask was associated with increased prevalence of dizziness, muscle pain, irritated nose, cough, nausea and blurred vision. We attempted to control for (exposure) selection bias by adjusting for day of cleanup that the workers worked. In contrast to mask use, boot or coverall use showed no consistent trends in sympton protection or exacerbation. To summarize, the overall evidence was not adequate to support the hypothesis that PPE use by Rayong oil spill workers was consistently protective against post-shift symptoms.

Table 4.6: Adjusted Odds Ratio* of Post-Shift Symptoms by PPE use (n=1,294)**

Post-shift Symptoms	Any PPE Use		Mask Use		Glove Use		Boot Use		Coverall Uses		Complete set of PPE	
	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI
Dizziness	1.30	(0.85 - 1.02)	**1.50**	**(1.03 - 2.21)**	1.02	(0.71 - 1.47)	1.05	(0.73 - 1.50)	1.06	(0.73 - 1.56)	1.18	(0.73 - 1.99)
Irritated Throat	**1.04**	**(1.01 - 1.10)**	1.25	(0.83 - 1.88)	0.70	(0.48 - 1.04)	0.95	(0.65 - 1.38)	0.82	(0.53 - 1.26)	0.63	(0.40 - 1.02)
Irritated Eyes	1.02	(0.97 - 1.07)	1.31	(0.87 - 1.99)	0.86	(0.58 - 1.28)	1.09	(0.74 - 1.61)	1.02	(0.67 - 1.54)	0.78	(0.49 - 1.29)
Muscle Pain	1.01	(0.94 - 1.05)	**1.69**	**(1.07 - 2.66)**	1.03	(0.66 - 1.61)	1.31	(0.87 - 1.98)	1.36	(0.83 - 2.22)	1.32	(0.74 - 2.54)
Irritated Nose	**1.05**	**(1.01 - 1.10)**	**1.86**	**(1.16 - 3.00)**	0.91	(0.59 - 1.42)	1.19	(0.78 - 1.83)	0.70	(0.44 - 1.12)	0.99	(0.57 - 1.83)
Sore Throat	1.00	(0.90 - 1.05)	0.96	(0.56 - 1.62)	1.04	(0.63 - 1.75)	1.44	(0.90 - 2.32)	1.42	(0.80 - 2.53)	0.62	(0.35 - 1.13)
Cough	**0.80**	**(0.64 - 0.94)**	**1.86**	**(1.03 - 3.37)**	0.75	(0.44 - 1.31)	**1.66**	**(1.00 - 2.81)**	1.85	(0.88 - 3.93)	0.70	(0.36 - 1.45)
Heavy Breathing	1.00	(0.88 - 1.06)	1.30	(0.76 - 2.23)	0.73	(0.44 - 1.22)	0.80	(0.48 - 1.31)	0.63	(0.36 - 1.10)	0.67	(0.38 - 1.26)
Running Nose	1.03	(0.96 - 1.08)	1.42	(0.78 - 2.60)	0.90	(0.52 - 1.59)	1.59	(0.94 - 2.70)	0.84	(0.40 - 1.71)	0.93	(0.46 - 2.09)
Skin Itching	1.02	(0.94 - 1.07)	1.52	(0.75 - 3.07)	1.04	(0.52 - 2.16)	0.94	(0.49 - 1.81)	1.08	(0.50 - 2.28)	1.03	(0.45 - 2.78)
Nausea	0.95	(0.75 - 1.06)	**2.57**	**(1.24 - 5.61)**	0.63	(0.32 - 1.24)	0.54	(0.26 - 1.07)	0.86	(0.42 - 1.73)	0.92	(0.42 - 2.32)
Blurred Vision	1.00	(0.85 - 1.06)	**2.88**	**(1.28 - 6.86)**	0.84	(0.39 - 1.82)	1.01	(0.48 - 2.12)	1.39	(0.62 - 3.11)	1.36	(0.52 - 4.69)
Feeling Faint	1.03	(0.88 - 1.10)	1.19	(0.49 - 2.90)	0.90	(0.40 - 2.08)	1.08	(0.49 - 2.38)	0.75	(0.28 – 2.00)	0.96	(0.36 - 3.35)
Abdominal Pain	0.97	(0.73 - 1.06)	0.96	(0.34 - 2.43)	1.07	(0.44 - 2.86)	1.39	(0.61 - 3.27)	0.86	(0.25 - 2.63)	0.81	(0.29 - 2.86)
Eye Tearing	1.04	(0.94 - 1.09)	1.58	(0.64 - 3.92)	0.57	(0.24 - 1.34)	0.85	(0.67 - 1.95)	3.22	(1.18 - 9.80)	0.83	(0.28 - 2.25)
Diarrhea	**0.47**	**(0.18 - 0.91)**	0.61	(0.16 - 1.81)	0.98	(0.38 - 2.85)	1.48	(0.60 - 3.82)	**0.17**	**(0.01 - 0.92)**	0.93	(0.30 - 4.05)
Eye Injection	1.05	(0.98 - 1.11)	0.55	(0.18 - 1.50)	0.34	(0.12 - 0.88)	0.37	(0.12 - 0.98)	0.38	(0.10 - 1.14)	**0.17**	**(0.07 - 0.44)**
Eczema	0.99	(0.77 - 1.06)	1.37	(0.49 - 3.66)	1.00	(0.37 - 2.96)	1.69	(0.67 - 4.45)	1.03	(0.33 - 2.88)	0.98	(0.32 - 4.27)
Chest Tightness	**0.64**	**(0.33 - 0.99)**	1.77	(0.62 - 5.34)	0.59	(0.21 - 1.68)	0.68	(0.23 - 1.87)	0.87	(0.28 - 2.56)	0.95	(0.30 - 4.19)
Palpitation	1.02	(0.82 - 1.09)	1.26	(0.45 - 3.69)	0.76	(0.27 - 2.12)	0.52	(0.16 - 1.45)	0.90	(0.31 - 2.51)	0.71	(0.24 - 2.60)
Injuries	0.79	(0.39 - 1.06)	0.56	(0.12 - 2.05)	1.02	(0.31 - 3.95)	2.40	(0.76 - 9.02)	0.18	(0.01 - 1.06)	0.54	(0.16 - 2.47)
Taste Change	0.98	(0.57 - 1.12)	4.00	(0.81 - 30.45)	1.24	(0.29 - 6.24)	1.85	(0.44 - 9.16)	0.84	(0.16 - 3.94)	1.58	(0.27 - 29.95)
Vomiting	Sample size is too small to calculate odds ratio											

*** Odds ratio adjusted by urinary cotinine and day of cleanup.**
****49 workers had at least one piece of missing data** **Bold** numbers indicate statistically significant results (P<0.05).

Exploratory Factor Analysis

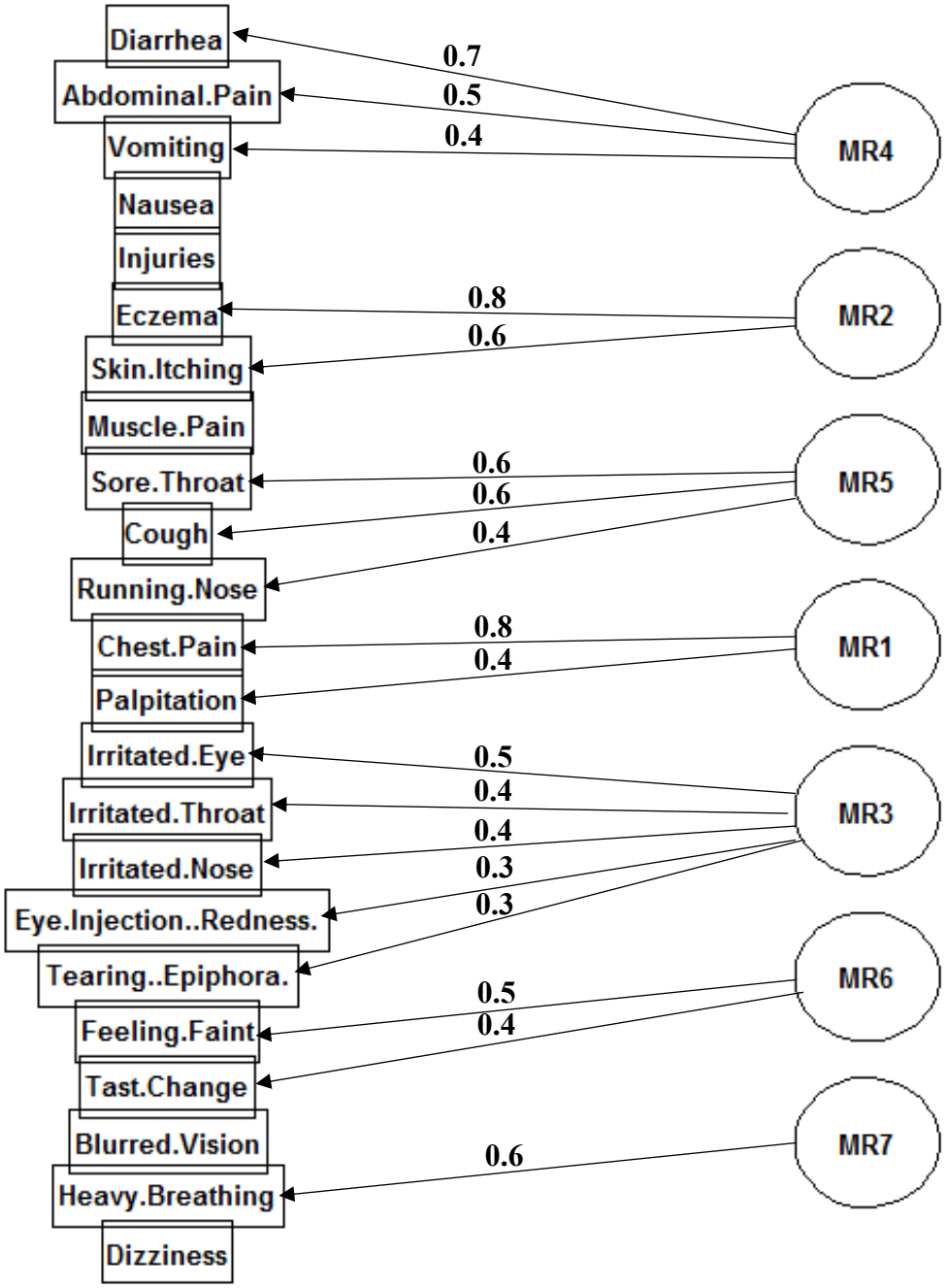
An exploratory factor analysis was performed in order to examine the hypothesis that some of these symptoms might share underlying common pathologies and be highly correlated in groups. As described earlier, the optimal number of factors (groups) was 7, and post-shift symptoms were grouped by factor analysis as shown in Table 4.7 and Figure 4.5.

Table 4.7: Groups of Symptoms Determined by Factor Analysis

Group	Symptoms	Symptom Group Name
MR1	**Chest tightness, Palpitation**	Chest Symptoms
MR2	**Urticaria, Skin Itching**	Allergic Skin Reaction
MR3	**Irritation of Eye, Throat, Nose, Eye injection (Redness), Eye Tearing (Epiphora)**	Irritative Symptoms
MR4	**Diarrhea, Abdominal Pain, Vomiting**	Gastrointestinal Symptoms
MR5	**Sore Throat, Running Nose, Cough**	Respiratory Tract Symptoms
MR6	**Feeling Faint, Taste Changes**	Sensation Errors
MR7	**Heavy Breathing**	Heavy Breathing

*MR = Minimun Residual; In our exploratory factor analysis, we used the Ordinary Least Squares (OLS) method to find the minimum residual solution for the EFA.

Figure 4.5: Factor Analysis Diagram showing the EFA results (Correlation coefficients) of 23 Post-Shift Symptoms



Factor Analysis on Post-Shift Symptoms and Urinary Metabolites (1-OHPG and t,t-MA)

The results from ordinal logistic regression on group symptoms indicated that the irritative symptoms were associated with the level of urinary 1-OHPG in workers. The adjusted odds ratio of irritative symptoms, a symptom group consisting of irritated eye, throat and nose, eye injection (redness) and excessive tearing (epiphora), was 1.04 (95%CI: 1.01 - 1.07) per 1 pmol/ml increase in urinary 1-OHPG concentration (Table 4.8). On the other hand, detectable urinary t,t-MA was not statistically associated with any symptom group (Table 4.9).

Table 4.8: Adjusted Odds Ratio of Symptom Groups (per 1 pmol/ml increase in 1-OHPG) from Ordinal Logistic Regression and Factor Analysis (n=1,318)

Symptom Group	MR	Crude OR		Adjusted OR*	
		OR	95% CI	OR	95% CI
Chest Symptoms	**1**	0.98	(0.79 - 1.06)	0.98	(0.77 - 1.06)
Allergic Skin Reaction	**2**	1.02	(0.93 - 1.07)	1.02	(0.94 - 1.06)
Irritative Symptoms	**3**	**1.03**	**(1.00 - 1.06)**	**1.04**	**(1.01 - 1.07)**
Gastrointestinal Symptoms	**4**	0.84	(0.62 - 1.02)	0.89	(0.64 - 1.04)
Respiratory Tract Symptoms	**5**	0.96	(0.88 - 1.02)	1.00	(0.93 - 1.05)
Sensation Errors	**6**	1.01	(0.88 - 1.07)	1.03	(0.89 - 1.09)
Heavy Breathing	**7**	0.99	(0.88 - 1.05)	1.00	(0.88 - 1.06)

***Odds ratio adjusted by urinary cotinine and days of cleanup**

Bold numbers indicate statistically significant results. ($P<0.05$)

Table 4.9: Adjusted Odds Ratio of Symptom Groups (by detectable vs non-detectable t,t-MA) from Ordinal Logistic Regression and Factor Analysis (n=1,318)

Symptom Group	Crude OR		Adjusted OR*	
	OR	95% CI	OR	95% CI
Chest Symptoms	0.65	(0.23 - 1.54)	0.54	(0.18 - 1.43)
Allergic Skin Reaction	1.06	(0.55 - 1.97)	1.10	(0.53 - 2.23)
Irritative Symptoms	0.93	(0.70 - 1.24)	1.01	(0.73 - 1.37)
Gastrointestinal Symptoms	1.48	(0.74 - 2.89)	1.51	(0.67 - 3.33)
Respiratory Tract Symptoms	0.84	(0.58 - 1.20)	0.81	(0.53 - 1.23)
Sensation Errors	1.14	(0.52 - 2.35)	1.02	(0.43 - 2.29)
Heavy Breating	1.23	(0.74 - 2.02)	1.31	(0.75 - 2.24)

***Odds ratio adjusted by urinary cotinine and days of cleanup**

Bold numbers indicate statistically significant results. (P<0.05)

Factor Analysis on Post-Shift Symptoms and Job Descriptions

Due to the relatively small sample size, ordinal logistic regression could not be performed for the analysis of symptoms by job description. Therefore, we assessed the association between symptom groups (having at least one symptom in the group) and job descriptions using simple logistic regression. The results from the simple logistic regression on symptom groups indicated (unexpectedly) that the prevalence of heavy breathing was lower in contaminated sand/trash removal workers and oil vacuum/oil slick removal workers than in support personnel (Table 4.10). The odds (95%CI) of heavy breathing were 0.34 (0.15 - 0.87) in contaminated sand/trash removal workers and 0.30 (0.11 - 0.84) in oil vacuum/oil slick removal workers, compared to support personnel.

Table 4.10: Adjusted Odds Ratio of Symptom Groups by Job Descriptions from Simple Logistic Regression and Factor Analysis (n=1,150)

Symptom Group	Support Personnel	Contaminated Sand/ Trash Removal		Oil Vacuum/ Oil Slick Removal	
	OR*	OR*	(95% CI)	OR*	(95% CI)
Chest Symptoms	1.00 (Ref)	NA		NA	
Allergic Skin Reaction	1.00 (Ref)	0.37	(0.11 - 1.74)	0.82	(0.23 - 3.92)
Irritative Symptoms	1.00 (Ref)	0.56	(0.31 - 1.05)	0.73	(0.38 - 1.40)
Gastrointestinal Symptoms	1.00 (Ref)	1.05	(0.18 - 20.34)	1.70	(0.26 - 33.7)
Respiratory Tract Symptoms	1.00 (Ref)	0.64	(0.29 - 1.57)	0.77	(0.33 - 1.97)
Sensation Errors	1.00 (Ref)	1.00	(0.18 - 18.94)	2.38	(0.41 - 45.67)
Heavy Breathing	1.00 (Ref)	**0.34**	**(0.15 - 0.87)**	**0.30**	**(0.11 - 0.84)**

***Odds ratio adjusted by urinary cotinine and days of cleanup**

NA due to too small sample size to calculate Odds Ratio. 0/60 support personnel had Chest tightness and palpitation symptoms.

Bold numbers indicate statistically significant results. (P<0.05)

Factor Analysis on Post-Shift Symptoms and PPE Use

Personel protective equipment use did not appear to be related to group symptoms, with the exception of mask use being positively associated with irritative symptoms (Table 4.11).

Table 4.11: Adjusted Odds Ratio of Symptom Groups by PPE Use from Ordinal Logistic Regression and Factor Analysis (n=1,294)

Group of Symptoms	Any PPE USE		Mask Use		Glove Use		Boot Use		Coverall Uses		Complete set of PPE	
	OR	**95%CI**	**OR**	**95%CI**	**OR**	**95%CI**	**OR**	**95%CI**	**OR**	**95%CI**	**OR**	**95%CI**
Chest Symptoms	1.00	(0.37 - 3.49)	2.17	(0.93 - 5.40)	0.73	(0.32 - 1.68)	0.74	(0.32 - 1.66)	1.05	(0.44 - 2.44)	1.18	(0.42 - 2.88)
Allergic Skin Reaction	1.27	(0.53 - 3.76)	1.36	(0.69 - 2.63)	1.21	(0.62 - 2.48)	1.13	(0.61 - 2.09)	1.01	(0.49 - 2.03)	1.03	(0.41 - 2.28)
Irritative Symptoms	0.92	(0.62 - 1.41)	**1.36**	**(1.01 - 1.84)**	0.77	(0.58 - 1.03)	1.13	(0.86 - 1.50)	0.88	(0.65 - 1.20)	1.08	(0.74 - 1.54)
Gastrointestinal Symptoms	0.72	(0.31 - 1.99)	0.68	(0.27 - 1.55)	1.23	(0.58 - 2.87)	1.48	(0.74 - 3.03)	0.52	(0.16 - 1.38)	1.02	(0.29 - 2.86)
Respiratory Tract Symptoms	0.78	(0.48 - 1.30)	1.19	(0.79 - 1.78)	0.97	(0.66 - 1.42)	1.54	(1.08 - 2.19)	1.28	(0.83 - 1.99)	1.23	(0.73 - 2.02)
Sensation Errors	2.09	(0.61 - 13.14)	1.65	(0.73 - 3.80)	0.87	(0.41 - 1.89)	1.10	(0.53 - 2.30)	0.83	(0.34 - 2.00)	1.39	(0.49 - 3.49)
Heavy Breathing	0.84	(0.44 - 1.80)	1.30	(0.76 - 2.23)	0.73	(0.44 - 1.22)	0.80	(0.48 - 1.31)	0.63	(0.36 - 1.10)	1.07	(0.54 - 1.99)

***Odds ratio adjusted by urinary cotinine and days of cleanup**

Bold numbers indicate statistically significant results. (P<0.05)

DISCUSSION

In this study of Rayong oil spill cleanup workers, we examined prevalence of post-shift acute symptoms among the workers, and assessed their association with potential causative factors, including internal dose biomarkers of PAH and benzene exposure, day of cleanup worked, job description, PPE use, and age of workers. We further examined causative factors associated with groups of correlated symptoms determined by factor analysis. Prevalence of some post-shift symptoms, including irritation of throat and nose, increased with concentration of urinary 1-OHPG, an internal dose biomarker of PAHs. Similarly, one group of symptoms designated as "irritative symptoms", including irritation of the eye, throat and/or nose, eye injection (redness) and excessive tearing (epiphora), was also associated with increased concentration of urinary 1-OHPG. After adjusting for cotinine and day of cleanup worked, this association remained significant. In occupational settings, PAHs have been reported to be related to irritation of the chest and throat.[23] Our study suggests that the levels of PAHs (or other compounds) present at oil spill sites are sufficient to cause irritative symptoms in cleanup workers. The association between 1-OHPG and irritative symptoms might be due to related effects from the bioaerosols and particulate matter to which the PAHs are bound.[24]

In the current study, none of the physical symptoms reported by workers were associated with urinary t,t-MA, an internal dose biomarker of benzene. However, this may be due to the relatively low proportion of workers with detectable urinary t,t-MA in our study, and is consistent with the knowledge that benzene causes acute symptoms only at relatively high concentrations in air ($\geq$ 50 ppm).[15, 16] Previous studies from the Hebei oil spill examined the association between post-shift symptoms and biomarkers of PAHs in urine, as well as biomarkers of benzene, toluene, ethyl benzene, and xylene (BTEX) (Cheong et al, 2011 and Ha et al, 2012).[23, 25] They reported that only musculoskeletal symptoms were associated with concentration of urinary 1-hydroxypyrene (1-OHP) (Cheong et al.)[25], and only dermal irritation was associated with concentration of urinary t,t-MA (Ha et al).[23] In contrast to our study, none of the reported irritative symptoms were found

to be associated with urinary 1-OHP.[23, 25] However, they found that nasal irritation, nausea sensation, vomiting, fatigue and fever were associated with other BTEX biomarkers, including urinary hippuric and methyl-hippuric acid.[25] The study of Peres et al (2016) from the Deepwater Horizon oil spill found that a burning sensation in the nose, throat or lungs, sore throat, dizziness and wheezing, were strongly associated with an exposure category by factor analysis of "high physical-environmental exposure" (including cleanup participation and property lost due to the oil spill) in adult women (overall n=2126, high n = 453).[26]

Several factors may have contributed to the variation in self-reported symptoms and their association with internal dose biomarkers. Post-shift symptoms were self-reported and subjective. People in different cultures and settings may perceive and interpret symptoms caused by the same pathophysiology, in a different manner, such as hypoglycemia[27] and menopause[28], even in the same country. Another complicating factor is the lack of standardization of symptom questionnaires for oil spill cleanup workers, adding to variability in question phrasing and symptoms emphasized.

Physical symptoms and their determining factors were reported differently among various studies that assessed symptoms among oil spill cleanup workers. In our study, 36.3% of workers reported having at least one of 23 different symptoms, with the most prevalent symptom being dizziness (10.7%). Another study of workers at the Rayong oil spill reported by Sithisarankul et al, (2015), [29] found that the five organ systems with the highest prevalence of symptoms were pulmonary (38.6%), integumentary and mucous membranes (17.0%), neurological (16.9%), musculoskeletal (7.7%) and gastrointestinal (6.1%) (n = 2,096).[29] The study of Meo et al (2009) from the Tasman Spirit oil spill reported prevalence of symptoms among cleanup workers ranging from 18% for general illnesses to 38% for cough, and these prevalences were much higher than among matched controls. The two studies from the Hebei oil spill reported the prevalence of post-shift symptoms as 36-47% (Ha et al)[23] and 37-90% (Cheong et al)[25] in the participants. The Hebei studies combined 41 symptom questions into 14 categories, reporting prevalence of categories rather than individual symptoms. The study of D' Andrea and Reddy (2013) found that the top 3

symptoms reported by Deepwater Horizon oil spill workers were headaches (75%), shortness of breath (71%) and skin rash (61%) (n=117). The size of the Rayong oil spill was relatively small with only about 50-300 barrels of oil released, much less than that of the Deepwater Horizon (5 million barrels)[30] or Hebei (80,000 barrels)[31] oil spills. This may explain the lower prevalence of symptoms reported among the Rayong workers. In addition, there may be differences in perception of symptoms in large versus small oil spills, artificially elevating symptom prevalence in large spills with expectation of high toxicant levels.

We also examined the prevalence of post-shift symptoms by days of cleanup. We found that prevalence of dizziness decreased in subsequent days/weeks of cleanup, compared to the first three days (day 2-4) of cleanup. This was consistent with our measure of internal dose of PAHs, urinary 1-OHPG, which decreased significantly over the 4 week course of the cleanup. Conversely, other symptoms did not decrease (or even increased) in subsequent days of cleanup, compared to day 2-4 of cleanup. Some of these symptoms (cough, runny nose and abdominal pain) may have been due to a possible outbreak of stomach flu among the military personnel in the latter weeks of cleanup. The study of Cheong et al. (2012) also explored the prevalence of symptoms by days of cleanup but did not find a decreasing trend as the cleanup progressed.[25]

Many factors, including those stated above, may have influenced the prevalence of symptoms, in addition to PAH exposure. For example, we also examined the prevalence of post-shift symptoms by age of workers, and found that workers aged 40 years and older reported higher prevalence of symptoms, including irritated throat, irritated eyes, irritated nose, blurred vision and red eye (eye injection), than younger workers. The older workers may have been more sensitive to physical labor or toxic chemicals, as has been suggested by studies of symptoms in construction workers[32] and white-collar workers.[33]

In our study, prevalence of symptoms also varied by job description in an unexpected pattern. For example, prevalence of irritated throat and nose, sore throat, and heavy breathing was highest in the support personnel (a group with lowest median levels of urinary 1-OHPG in our

exposure study), compared to oil contaminated sand/trash removal workers and oil vacuum/oil slick removal workers. Even after adjustment by age of workers, days of cleanup, and urinary cotinine levels, the prevalence of these symptoms was still significantly higher in the support personnel than in workers with job descriptions associated with higher potential oil exposure. These findings are complicated by the fact that most of the higher exposure jobs were performed by military personnel and PTTGC employees, whereas the support personnel consisted mostly of citizen volunteers. In addition to being younger, the military personnel and PTTGC employees may have been better informed and more thoroughly instructed regarding the potential hazards and safety procedures of oil spill cleanup work. This training may, in turn, have influenced their perception of the risks involved and related symptoms. Similar to our study, Ha et al[24] also found that the prevalence of eye and nose irritation, and headache was higher in logistics-related jobs, compared to direct cleanup jobs.

We also examined the association between post-shift symptoms and personal protective equipment (PPE) use. Wearing at least one piece of PPE appeared to be protective against three symptoms: cough, diarrhea, and chest tightness. Wearing coveralls appeared to be protective against diarrhea. However, the overall findings on the effectiveness of PPE use were not consistent. Other symptoms did not decrease in prevalence (or in some cases increased in prevalence) with use of PPEs, including mask, gloves, boots, or the complete set of PPEs. This suggests that not all PPEs were effective, or they were not used properly, or that the questionnaire data of PPE use and/or symptoms was not valid. Similar to our finding, Lee et al. (2009) found no evidence of protection by PPE use against post-shift symptoms, among workers at the Hebei oil spill.

Our study investigated PAH and benzene internal dose biomarkers in cleanup workers and their post-shift symptoms, ascertained within the first two days of a fresh oil spill. In contrast to studies of cleanup workers at the most intensively investigated spill, the Hebei oil spill, where urine sample collection started 2 weeks after cleanup started, our study assessed internal dose of PAHs and benzene beginning on the 2nd day of cleanup, at which time exposure was expected to be close

to maximum. In addition, we used factor analysis to categorize correlated groups of symptoms, rather than grouping symptoms by pre-defined categories or organ systems which may have resulted in misclassification and reduced statistical power. Furthermore, our study had a relatively large sample size (n=1343) compared to the three studies from the Hebei oil spill (n=121, n=154, n=724)[9,10,32]

The current study has several limitations. First, the statistical analysis was performed as a cross-sectional symptom analysis because most cleanup workers (88%) only worked one day, thus limiting our ability to assess causal inference. Second, post-shift symptoms were self-reported and somewhat subjective. The perceptions of workers (not captured by the questionnaire) toward the dangers of oil spill cleanup work, may have influenced their answers to the symptom questionnaire. Third, the study questionnaire and sampling strategy was initially designed as part of a health surveillance program initiated by the Rayong Provincial Health office, rather than a formal scientific study. As a result, some of the data, such as hours of cleanup participation, smoking status, dietary patterns, and pre-exposure assessment was not complete or unavailable for statistical analysis. Fourth, our study did not have an ideal negative control population that was unexposed to crude oil, such as pre-cleanup baseline measurements of workers, or non-participants who were not involved in the cleanup. For these reasons, it is difficult to assess the magnitude of the increase in prevalence of post-shift symptoms.

This study will form the foundation for the health followup and survelliance of these workers at Rayong Hospital, which is ongoing and planned to last 5 years. In future studies, biomarkers of oxidative damage, such as urinary malondialdehyde (MDA), that has been associated with PAH exposure in several studies[34, 35] might be useful in understanding the association between acute symptoms and PAHs. Additional studies are needed to explore the occurrence and persistence of symptoms among oil spill cleanup workers with complementary exposure data. There is a need for standardized symptom questionnaires designed for oil spill cleanup workers, as well as standardized sample collection strategies.

In conclusion, Rayong oil spill cleanup workers exhibited evidence of an association between prevalence of acute irritative symptoms and PAH exposure measured by urinary 1-OHPG. Long-term health monitoring of oil spill cleanup workers should be implemented, particularly among those workers suspected of sustaining high exposure to crude oil. Standard health guidelines and symptom questionnaires for oil spill cleanup worker should be established internationally.

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CHAPTER 5: CONCLUSIONS

The goal of this dissertation was to determine if Rayong oil spill cleanup workers were exposed to elevated levels of PAHs and benzene, and determine if these exposures were associated with recorded acute symptoms. To address this goal, the urinary concentrations of 1-OHPG were measured in the 1,343 available frozen stored urine samples from the workers, and the previously measured urinary concentrations of t,t-MA were re-examined. To assess possible factors related to PAH and benzene exposure, the health, work, and demographic questionnaire data was linked to the measured concentrations of 1-OHPG and t,t-MA. Finally, the post-shift acute symptom data was linked to the aforementioned dataset to assess the association between acute symptoms and the internal dose biomarkers. The results and conclusions are described in the context of our specific aims.

Specific Aim 1: To determine the internal dose of PAHs and benzene among 1,343 Rayong oil spill cleanup workers by measuring the PAH biomarker 1-OHPG in previously collected urine samples and re-examining the t,t-MA concentrations previously measured.

In the first manuscript, Chapter 3, we reported elevated levels of urinary 1-OHPG among oil spill workers who worked during the first few days of the cleanup, when potential exposures to crude oil were high. These levels of 1-OHPG are similar to those reported in occupational settings with high PAH exposures, such as in steel plant workers (Kang et al 1995)[1]. For comparison, the observed levels of 1-OHPG in our workers were much higher than those observed among non-smokers in the U.S. or in Thailand.[2] We reported a high percentage (67.5%) of samples with nondetectable concentrations of urinary t,t-MA in our study. However, the method previously used to measure t,t-MA in these workers[3] had limited sensitivity, with an estimated limit of quantification of about 0.10 ug/ml. This is comparable to background t,t-MA levels in the general population of 0.07 μg/ml (range: 0.02-0.30 μg/ml).[4, 5] Therefore, we can only conclude that about 30% of the workers, whose urinary t,t-MA levels were detectable, had benzene exposures

comparable to the top quartile of that experienced by the general U.S. population. In conclusion, the Rayong oil spill cleanup workers were exposed to PAHs at elevated levels, comparable to occupational exposures in high PAH exposure settings, whereas benzene exposures among the oil spill workers fell within the general population range.

Specific Aim 2: To compare the internal dose of workers who worked on different days of cleanup: early week 1 (day 2-4), late week 1 (day 5-7), week 2, week 3 and week 4, adjusting for smoking status (cotinine) and to examine factors related to their dose, including personal protective equipment (PPE) use and job descriptions.

We reported that the elevated levels of urinary 1-OHPG among oil spill workers who worked during the first few days of cleanup (0.97 pmol/ml) declined to 0.32 pmol/ml in workers who worked 4 weeks later (days 22-28). Thus, the 1-OHPG levels we observed were comparable to occupational exposures during the early days of cleanup, and declined to near background (general population) levels by the end of the cleanup operations.

We also examined the levels of urinary 1-OHPG among cleanup workers with different job descriptions. We found that certain types of jobs including, oil dispersant applicators, contaminated sand/trash removal workers and oil vacuum/oil slick removal workers, had higher levels of urinary 1-OHPG than other workers and support personnel. Oil dispersant applicators might be at increased risk of PAH exposure because spraying dispersants on oil-water interfaces generates aerosols that are respirable (Ehrenhauser et al, 2013). Water wave action on the sea while applying dispersants can also facilitate aerosolization and evaporation of PAHs (Ehrenhauser et al, 2013). Workers dealing with contaminated sand/trash removal and oil vacuum/oil slick removal were often in close (or direct) contact with crude oil, thereby enhancing the possibility of dermal contamination. These findings expand our knowledge of the exposure profiles of oil spill cleanup workers with respect to specific job descriptions.

The effect of PPE use on PAH exposure among cleanup workers was investigated, but levels of urinary 1-OHPG were not associated with overall PPE use, consistent with the finding of Lee et al. (2009) from Hebei oil spill.[6] Furthermore, individual equipment use (masks, gloves, boots, or coveralls) was not associated with a protective effect. This suggests that PPE was not effective, or was not used properly, or that the questionnaire data was not reliable. Alternatively, this might have resulted from exposure selection bias because of higher hazard recognition (resulting in enhanced PPE use) in the early days of cleanup when the beach was covered in oil, compared to later weeks of cleanup.

This is the first investigation of PAH biomarkers in cleanup workers' urine samples that were collected within two days of a fresh oil spill. Furthermore, the sample size of our study (n=1343) is relatively large, compared to other studies from the Hebei oil spill[9,10,32] and the Deepwater Horizon oil spill[7]. In the current study, we also used urinary cotinine to adjust for expected confounding effects of smoking on PAH biomarkers, which were not used in previous studies. These new findings confirm the assumption that oil spill cleanup workers are exposed to significantly elevated levels of PAHs, especially during the first days of a spill, and that PAH exposure declines within a few weeks of cleanup following a relatively small spill.

In addition to the cleanup process, a number of other factors could contribute to differences in PAH exposure patterns between spills and between studies. The half-life of PAHs in crude oil in the environment can range from a few hours up to weeks or months depending on the chemical composition of the oil, the molecular weights of the PAHs, bacterial biodegradation and photolysis.[8, 9] After a spill and during cleanup, low molecular weight PAHs would be expected to evaporate within a few days, resulting in the rapid decline in biomarkers, while the higher molecular weight PAHs might take a few weeks to evaporate or degrade. Pyrene, the parent compound of 1-OHPG, is of intermediate MW (m=202) having both rapid and slow evaporation characteristics.

We also found that the percentage of oil spill workers with detectable urinary t,t-MA decreased from day 2-4 to week 3-4 of cleanup in nonsmokers, but not in smokers. Because a high

percentage (67.5%) of samples had nondetectable levels of t,t-MA in our study, we analyzed the t,t-MA data as a binary variable – detectable vs nondetectable.. This limited our statistical power to detect associations[10] and might partially explain why we did not observe a decreasing trend of urinary t,t-MA detectable percentage with time of cleanup in smokers. In addition, tobacco smoke contains benzene and significantly increases the concentration of urinary t,t-MA in smokers compared to nonsmokers.[11] Compared to 1-OHPG, the percentage of detectable t,t-MA samples among nonsmokers decreased more rapidly with days of cleanup than the decline in levels of 1-OHPG. The rapid decline in detectable t,t-MA, is not unexpected as benzene is relatively more volatile than pyrene, and would be expected to evaporate within a few days of the oil spill.

These findings contribute to our knowledge of possible benzene exposure among oil spill cleanup workers. The observation that urinary t,t-MA declined over the course of the cleanup among non-smokers strongly suggests that the workers did experience elevated benzene exposure in the early days of the cleanup. Urinary t,t-MA measured in other studies of oil spill workers is somewhat conflicting. Ha et al (2012) found that levels of urinary t,t-MA of workers at the Hebei spill were higher after cleanup participation compared to levels before participation among both smokers and nonsmokers,[12] whereas, in another group of Hebei spill cleanup workers, Cheong et al (2011) reported no difference in t,t-MA levels between workers and unexposed controls.

Specific Aim 3: To examine the association between levels of internal dose biomarkers and acute symptoms previously recorded.

In the second manuscript, Chapter 4, the prevalence of post-shift acute symptoms among the oil spill workers and their associations with potential causative factors, including internal dose biomarkers of PAH and benzene exposure, were explored and assessed. We further examined causative factors associated with groups of correlated symptoms determined by factor analysis.

Prevalence of some post-shift symptoms, including irritation of throat and nose, increased with concentration of urinary 1-OHPG. Similarly, one group of symptoms designated as “irritative

symptoms" in factor analysis, including irritation of the eye, throat and/or nose, eye injection (redness) and excessive tearing (epiphora), was also associated with increased concentration of urinary 1-OHPG. Our study suggests that the levels of PAHs (or other compounds) present at oil spill sites are sufficient to cause irritative symptoms in cleanup workers. On the other hand, none of the physical symptoms reported by workers were associated with urinary t,t-MA. This may be due to the relatively low proportion of workers with detectable urinary t,t-MA in our study, and is consistent with the knowledge that benzene causes acute symptoms only at relatively high concentrations in air ($\geq$ 50 ppm).[15, 16] While previous studies from the Hebei oil spill reported that musculoskeletal symptoms were associated with urinary 1-hydroxypyrene (Cheong et al.)[13], and dermal irritation was associated with concentration of urinary t,t-MA (Ha et al),[12] our study is the first to show an association between a group of correlated irritative symptoms and internal dose of PAHs.[12, 13]

We also examined the prevalence of post-shift symptoms by days of cleanup. We found that prevalence of dizziness decreased in subsequent days/weeks of cleanup, compared to the first three days (day 2-4) of cleanup. This was consistent with our measure of internal dose of PAHs, urinary 1-OHPG, which decreased significantly over the 4 week course of the cleanup. Conversely, other symptoms did not decrease (or even increased) in subsequent days of cleanup, compared to day 2-4 of cleanup. Many factors, including those stated above, may have influenced the prevalence of symptoms, in addition to PAH exposure. For example, we examined the prevalence of post-shift symptoms by age of workers, and found that workers aged 40 years and older reported higher prevalence of symptoms, including irritated throat, irritated eyes, irritated nose, blurred vision and red eye (eye injection), than younger workers.

In our study, prevalence of symptoms also varied by job description in an unexpected pattern. For example, prevalence of irritated throat and nose, sore throat, and heavy breathing was highest in the support personnel (a group with the lowest median level of urinary 1-OHPG in our exposure study), compared to oil contaminated sand/trash removal workers and oil vacuum/oil slick

removal workers. These findings are complicated by the fact that most of the higher exposure jobs were performed by military personnel and PTTGC employees, whereas the support personnel consisted mostly of citizen volunteers. In addition to being younger, the military personnel and PTTGC employees may have been better informed and more thoroughly instructed regarding the potential hazards and safety procedures of oil spill cleanup work. This training may, in turn, have influenced their perception of the risks involved and related symptoms.

We also examined the association between post-shift symptoms and personal protective equipment (PPE) use. The overall findings on the effectiveness of PPE use were not consistent. Although wearing at least one piece of PPE appeared to be protective against cough, diarrhea, and chest tightness, other symptoms did not decrease in prevalence (or in some cases increased in prevalence) with use of PPEs, including mask, gloves, boots, or the complete set of PPEs. This finding is analogous to our earlier observation that PPE use did not appear to influence internal PAH dose among the workers, but we cannot rule out the possibility that the PPEs were not used properly, or that the questionnaire data of PPE use and/or reported symptoms were not valid.

PUBLIC HEALTH IMPLICATION AND FUTURE RESEARCH

Since fossil fuels remain a major source of energy, the probability of oil spills and their consequences to human health and the environment, persists. A prudent strategy to deal with these unwanted events may be to have an emergency response plan in place prior to spills occurring. In addition to efforts to contain and remove oil-contaminated debris from the environment, such a plan should consider the health of people residing near the spill site and workers participating in the cleanup.

Many health and exposure studies of oil spill cleanup workers have severe limitations due to the fact that oil spills are unpredictable, thereby resulting in studies designed and implemented on very short notice. Exposure and health assessment instruments are rarely in place during the critical early days of an oil spill response. Most studies are cross-sectional in design and seldom have pre-exposure baseline data, thus limiting their ability to assess causal inference. Investigations of health effects following an oil spill often use self-reported symptoms that are subjective and can be unreliable. For these reasons, it is difficult to assess the magnitude of an increase in exposure or prevalence of post-shift symptoms and causal inference.

Our study emphasizes the risk of exposure to PAHs in oil spill cleanup workers, especially during the first days and weeks of cleanup. The evidence for exposure to benzene among the workers is less compelling, but suggests that some of the workers may have experienced benzene exposures during the early days of cleanup. Based on our results, the current health surveillance plan of the Rayong hospital will focus on the oil spill cleanup workers who experienced the highest PAH exposures, based on internal dose measurements during the first week of cleanup, that is, oil vacuuming/oil slick removal workers and sand/trash removal workers. This plan is currently supported by the Thai government for a period of up to 5 years. Because benzene and some PAHs are carcinogens, it may be prudent to continue to follow these workers, and others similarly exposed, for periods longer than 5 years. Long term surveillance could be passive, using the Thai national cancer registry.

Since our worker symptom results showed that only irritative symptoms were associated with PAH exposure, it may be necessary to include more objective measurements of early effects, such as biomarkers of oxidative stress, e.g., urinary malondialdehyde (MDA). These biomarkers have been associated with PAH exposure in several studies,[14, 15] and could prove useful in understanding the association between acute symptoms and PAHs. There is also a need for standardized symptom questionnaires designed for oil spill cleanup workers, as well as standardized sample collection strategies. Standard health guidelines and symptom questionnaires for oil spill cleanup workers should be established internationally.

There are also significant ethical issues associated with conducting epidemiological research following public health emergencies, such as oil spills. For example, Resnick et al[16] have examined a variety of ethical issues encountered in the GuLF Study following the Deepwater Horizon spill, including obtaining valid informed consent on short notice, expediting IRB review, and preparing for future events. They suggest "developing protocols, consent forms, survey instruments, and other documents prior to the advent of a public health emergency to allow for adequate and timely review." This would allow for more rapid implementation of health related studies.

Regarding future studies, the optimal study design would be a longitudinal study assessing worker exposure and health consequences over the course of their participation in an oil spill cleanup. Although difficult to implement on short notice, this approach would allow the exploration of temporal exposure and outcome patterns. In addition, biomarkers of internal dose and early biological effects should be used to monitor exposure and health outcomes over time. Improved biomarkers for benzene exposure, such as, urinary S-phenyl mercapturic acid, which is more specific for benzene than t,t-MA, could be used in future studies. Also, genetic polymorphisms in phase I enzymes, such as CYP1A1 and CYP1B1[17], and phase II enzymes, such as glutathione S-transferases, N-acetyltransferase-1, and epoxide hydrolase, might explain some of the variation in levels of internal dose and symptoms that we observed.

Additional studies are needed to explore the occurrence and persistence of symptoms among oil spill cleanup workers with complementary exposure data. Although several studies, including ours, reported that PPE use did not appear to reduce internal PAH or benzene dose among the workers, we cannot rule out the possibility that workers were subject to exposure recognition bias, or that the PPEs were not used properly. Therefore rigorous PPE training and cleanup preparedness for workers, as well as other volunteers, should be implemented before participation in oil spill cleanup activities.

SUMMARY

Rayong oil spill cleanup workers exhibited evidence of elevated levels of PAH and benzene exposure during the early days of cleanup, compared to near background levels among workers during the fourth week of cleanup. Certain types of jobs including, oil dispersant applicators, contaminated sand/trash removal workers, and oil vacuum/oil slick removal workers, were at highest risk for PAH exposure. Prevalence of acute irritative symptoms were positively associated with PAH exposure measured by urinary 1-OHPG. Therefore, the Rayong oil spill cleanup workers may be at risk of developing adverse health consequences. Long-term health monitoring of oil spill cleanup workers should be implemented, particularly among those workers suspected of sustaining high exposure to crude oil. Although recommended guidelines for oil spill response procedures are well-established, guidelines for recruiting and health monitoring of oil spill cleanup workers is less developed. Standardized health guidelines, sample collection methods, and post-shift questionnaires for oil spill cleanup workers should be established nationally and internationally.

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APPENDICES

APPENDICES, CHAPTER 2

Appendix 2.1 Laboratory Analysis of Urinary 1-OHPG using Immunoaffinity Chromatography and Synchronous Fluorescence Spectroscopy (SFS)

Lab Protocol

1. **Acid Hydrolysis**
- 2 mls of urine + 0.2 ml of 1N HCL
- Incubate for 1 hr at 90 C using heating blocks

2. **Solid Phase (Extraction)**
- Apply C18 Sep-Pak catride with vacuum systems
- Prime with 4 ml of water, 4 ml of 30% methanol (in water), 4ml of 80% methanol (in water), 4 ml of water
- Load acid hydrolyzed sample by less than 1ml/min of flow rate
- Wash with 4 ml of water, 4 ml of 30% methanol (in water)
- Elute with 4 ml of 80% methanol (in water)

3. **Evaporating down eluted sample**
- Using Speed Vaccuum with heater on
- Watching the samples every 20 minutes twice, then every 15 minute
- Stop evaporating when 0.5 ml of volume left in the tube
- Add 1.5 mls of 0.5X PBS to each test tube and gently vortex
- Put reconstituted sample in heating block at 37 C for 20 minutes
- Add 2 mls of 0.5X PBS to each tube and vortex

4. **Imunoaffinity Chromatography**
- Immunoaffinity columns were prepared using poly-prep columns (0.8x4 cm) filled with CNBr-activated Sepharose4B (0.8 ml) coupled with monoclonal antibody 8E11, which recognizes PAH metabolites
- Prewash columns with 4 mls 0.5X PBS
- Load prepared samples
- Wash column with 4 mls 0.5X PBS
- Wash column with 4mls 35% MEOH/0.5X PBS Collect Fraction
- Wash column with 4mls 60% MEOH/0.5X PBS Collect Fraction
- Wash column with 4 mls 0.5X PBS
- Store column with 0.5X PBS
- Measure 1-OHPG in the final fraction (4mls) with SFS using large curvette

5. **SFS Reading**

Using Perkin Elmer LS-50B, Luminescence/Fluorescence Spectrophotometer, the wavelength difference was set at 34 nm, samples containing 1-OHPG possess a characteristic fluorescence excitation maximum at 347 nm with emission at 381 nm.

APPENDICES, CHAPTER 3

Appendix Table 3.1: Urinary 1-OHPG in Oil Spill Workers by Day of Work, Cotinine Concentration, and Demographic Factors

Variables	Description	Number of Workers	Urinary 1-OHPG (pmol/ml)			
			Geometric Mean	Median	1st Quartile	3rd Quartile
Total		1,343	0.72	0.79	0.31	1.81
Days of Cleanup						
Week 1.1	**Day 2-4**	537	0.88	0.97	0.42	2.14
Week 1.2	**Day 5-7**	328	0.68	0.75	0.29	1.79
Week 2	**Day 8-14**	282	0.72	0.76	0.29	1.92
Week 3	**Day 15-21**	115	0.59	0.60	0.29	1.33
Week 4	**Day 22-28**	81	0.28	0.32	0.16	0.74
Cotinine Categories (ng/ml)						
1st Quartile	**ND-3.0**	334	0.54	0.58	0.27	1.37
2nd Quartile	**3.0-37.3**	337	0.57	0.58	0.28	1.37
3rd Quartile	**37.3-1,230**	336	0.55	0.58	0.26	1.39
4th Quartile	**1,230-3,177**	336	1.53	1.65	0.87	3.02
Nonsmoker	**<=50.0**	679	0.55	0.58	0.27	1.37
Smokers	**> 50.0**	664	0.93	1.07	0.45	2.28
Demographic Factors						
Sex	**Male**	1252	0.71	0.78	0.31	1.82
	Female	90	0.77	0.88	0.36	1.60
Age	**20-30**	772	0.65	0.70	0.30	1.61
	>30	557	0.82	0.91	0.34	2.04
Background Occupation						
- **Military Personnel**		743	0.63	0.66	0.29	1.59
- **PTTGC Employee**		402	0.78	0.91	0.35	1.94
- **Citizen Volunteer**		189	1.00	1.19	0.41	2.46

Appendix Table 3.2: Urinary 1-OHPG in Oil Spill Workers by Days of Cleanup Stratified by Smoking Status (Based on Cotinine Concentration of 50 ng/ml)

Variables	Description	Urinary 1-OHPG (pmol/ml)				
		Number	Geometric Mean	Median	1st Quartile	3rd Quartile
Total		1,343	0.72	0.79	0.31	1.81
Nonsmoker	**<=50.0 ng/ml of cotinine**	679	0.55	0.58	0.27	1.37
Week of Study	**Days of Cleanup**					
Week 1.1	**Day 2-4**	346	0.73	0.81	0.36	1.71
Week 1.2	**Day 5-7**	150	0.50	0.58	0.24	1.36
Week 2	**Day 8-14**	122	0.44	0.47	0.23	0.89
Week 3	**Day 15-21**	32	0.33	0.31	0.25	0.62
Week 4	**Day 22-28**	31	0.14	0.18	0.03	0.32
Smokers	**> 50.0 ng/ml of cotinine**	664	0.93	1.07	0.45	2.28
Week of Study	**Days of Cleanup**					
Week 1.1	**Day 2-4**	191	1.24	1.40	0.54	2.89
Week 1.2	**Day 5-7**	180	0.86	0.95	0.37	2.33
Week 2	**Day 8-14**	160	1.05	1.37	0.57	2.64
Week 3	**Day 15-21**	83	0.73	0.78	0.44	1.47
Week 4	**Day 22-28**	50	0.43	0.57	0.22	0.96

Appendix Table 3.3: Urinary 1-OHPG by Personal Protective Equipment (PPE) Use (n=1294)*

PPE USE	Details	Urinary 1-OHPG (pmol/ml)				
		Number of Workers	Geometric Mean	Median	1st Quartile	3rd Quartile
Total		1,343	0.72	0.79	0.31	1.81
Missing		49	0.90	0.98	0.48	1.99
Any PPE USE	Yes	1,132	0.72	0.78	0.32	1.78
	No	162	0.71	0.80	0.28	2.03
Mask	Yes	603	0.80	0.87	0.37	1.97
(N95, R95)	No	691	0.64	0.71	0.28	1.65
Coverall	Yes	523	0.87	0.91	0.40	2.13
	No	771	0.62	0.70	0.28	158
Gloves	Yes	770	0.70	0.76	0.31	1.74
	No	524	0.74	0.80	0.32	1.89
Boots	Yes	589	0.67	0.72	0.31	1.89
	No	705	0.75	0.82	0.32	1.73
Frequency	Never	46	0.99	0.95	0.41	2.50
	Sometimes	426	0.70	0.80	0.31	1.75
	Often	737	0.71	0.76	0.31	1.81
	Missing	134	077	0.93	0.30	1.90

***49 workers had at least one piece of missing data**

Appendix Table 3.4: Urinary t,t-MA Detectable in Oil Spill Workers by Day of Work, Cotinine Concentration, and Demographic Factors

Variables	Description	Total (N)	Urinary t,t-MA				P-value*
			Detectable		Non-detectable		
			N	Percent	N	Percent	
Total		1,343	436	32.5	907	67.5	
Days of Cleanup							
Week 1.1	**Day 2-4**	537	161	30.0	376	70.0	0.460
Week 1.2	**Day 5-7**	328	112	34.1	216	65.9	
Week 2	**Day 8-14**	282	97	34.4	185	65.6	
Week 3-4	**Day 15-28**	196	66	33.7	130	66.3	
Cotinine Categories (ng/ml)							
1st Quartile	**ND-3.0**	334	71	21.3	263	78.7	**<0.001**
2nd Quartile	**3.0-37.3**	337	71	21.1	266	78.9	
3rd Quartile	**37.3-1,230**	336	76	22.6	260	77.4	
4th Quartile	**1,230-3,177**	336	218	64.9	118	35.1	
Non-Smoker	**<=50.0**	679	144	21.2	535	78.8	**<0.001**
Smokers	**> 50.0**	664	292	44.0	372	56.0	
Demographic Factors							
Sex	**Male**	1252	407	32.5	845	67.5	1.000
	Female	90	29	32.2	61	67.8	
Age	**20-30**	772	252	32.6	520	67.4	0.919
	>30	557	179	32.1	378	67.9	
Background Occupation							0.167
- **Military Personnel**		743	229	30.8	514	69.2	
- **PTTGC Employee**		402	131	32.6	271	67.4	
- **Citizen Volunteer**		189	74	39.2	115	60.8	

***P-value by chi-squared test**

Bold numbers indicate statistically significant results. (P<0.05)

Appendix Table 3.5: Urinary t,t-MA Detectable in Oil Spill Workers by Day of Work Stratified by Smoking Status (Based on Cotinine Concentration of 50 ng/ml)

		Urinary t,t-MA Detectable					
Weeks of Study	Days of Cleanup	Nonsmokers (Cotinine <=50 ng/ml)			Smokers (Cotinine >50 ng/ml)		
		Total	N Detectable	% Detectable	Total	N Detectable	% Detectable
Total		679	144	21.2	664	292	44.0
Week 1.1	**Day 2-4**	346	91	26.3	191	70	36.6
Week 1.2	**Day 5-7**	148	31	20.9	180	81	45.0
Week 2	**Day 8-14**	122	18	14.8	160	79	49.4
Week 3-4	**Day 15-28**	63	4	6.3	133	62	46.6
	P-Trend*	**0.001**			**0.090**		

***P-Trend by Kruskal-Wallis**

Bold numbers indicate statistically significant results. (P<0.05)

Appendix Table 3.6: Urinary t,t-MA Detectable in Oil Spill Workers by Job Description Stratified by Smoking Status (Based on Cotinine Concentration of 50 ng/ml)

	Urinary t,t-MA Detectable (>LOQ)					
Job Descriptions	**Nonsmokers (Cotinine <=50 ng/ml)**			**Smokers (Cotinine >50 ng/ml)**		
	Total	**Number >LOQ**	**% >LOQ**	**Total**	**Number >LOQ**	**% >LOQ**
Total	679	144	21.2	664	292	44.0
Oil Dispersant Applicator	6	0	0.0	11	5	45.5
Contaminated Sand/ Trash Removal	356	79	22.2	422	186	44.1
Environmental Sampling Personnel	8	0	0.0	1	1	100.0
Oil Vacuum/ Oil Slick Removal	170	35	20.6	145	56	38.6
Supervisor/Health Care Professional	29	5	17.2	9	5	55.6
Transport Driver/ Ship Pilot	11	3	27.3	12	8	66.7
Support Personnel*	42	9	21.4	19	12	63.2
Others	33	10	30.3	11	6	54.5
Missing	24	3	12.5	34	13	38.2

Appendix Table 3.7: Urinary t,t-MA Detectable in Oil Spill Workers by Personal Protective Equipment (PPE) Use

PPE Use	Details	Total (N)	Urinary t,t-MA				P-value*
			Detectable		Non-detectable		
			N	Percent	N	Percent	
Total		1,343	436	32.5	907	67.5	
Missing		49	15	3.4	34	3.7	
Any PPE USE	Yes	1,132	364	32.2	768	67.8	0.441
	No	162	57	35.2	105	64.8	
Mask	Yes	603	186	30.8	417	69.2	0.226
(N95, R95)	No	691	235	34.0	456	66.0	
Coverall	Yes	523	174	33.3	349	66.7	0.642
	No	771	247	32.0	524	68.0	
Gloves	Yes	770	257	33.4	513	66.6	0.433
	No	524	164	31.3	360	68.7	
Boots	Yes	589	195	33.1	394	66.9	0.688
	No	705	226	32.1	479	67.9	
Frequency	Never	46	15	32.6	31	67.4	0.744
	Sometimes	426	147	34.5	279	37.9	
	Often	731	231	31.3	506	118.8	

*** P-value by chi-squared test**

Appendix Table 3.8: Logistic Regression with GEE* of Detectable t,t-MA by Personal Protective Equipment (PPE) Use (n=1,294)**

Type of PPE	Odds Ratio of t,t-MA Detectable (95% CI)			
	Univariable Model	Model 1	Model 2	Model 3
Any PPE Use (vs No PPE use)	0.87 (0.62-1.24)	0.89 (0.63-1.28)	0.88 (0.60-1.29)	0.86 (0.56-1.34)
Mask (vs No Mask use)	0.87 (0.68-1.09)	0.92 (0.71-1.19)	0.95 (0.72-1.26)	0.99 (0.72-1.36)
Coverall (vs No Coverall use)	1.06 (0.83-1.34)	1.15 (0.88-1.50)	1.07 (0.80-1.43)	1.07 (0.77-1.48)
Gloves (vs No Gloves use)	1.10 (0.87-1.40)	1.06 (0.82-1.35)	1.03 (0.79-1.35)	0.95 (0.70-1.29)
Boots (vs No Boots use)	1.05 (0.83-1.32)	1.01 (0.80-1.29)	1.05 (0.81-1.36)	1.14 (0.85-1.53)
Frequency				
- Never	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
- Sometimes	1.09 (0.58-2.13)	1.07 (0.57-2.11)	1.18 (0.59-2.45)	1.36 (0.63-3.08)
- Often	0.94 (0.51-1.83)	0.93 (0.50-1.80)	1.00 (0.51-2.06)	1.19 (0.56-2.65)

Model 1: Adjusted by days of cleanup (day 2-4, day 5-7, day 8-14, day 15-21 and day 22-28)
Model 2: Adjusted by days of cleanup and urinary cotinine
Model 3: Adjusted by days of cleanup, urinary cotinine and creatinine

* Generalized Estimating Equation (GEE, Exchangeable Correlation Structure)
**49 workers had at least one piece of missing data

Bold numbers indicate statistically significant results. (P<0.05)

APPENDICES, CHAPTER 4

Appendix Table 4.1: Prevalence of Post-Shift Symptoms by Cotinine Levels (Less than or greater than 50 ng/ml)

Post-Shift Symptoms	Total		Nonsmokers (Cotinine ≤50 ng/ml)		Smokers (Cotinine > 50 ng/ml)		P-value*
	N	%	N	%	N	%	
Total Workers	1343	100.0	679	100.0	664	100.0	
Any Symptoms	487	36.3	242	35.6	245	36.9	0.553
Dizziness	142	10.6	75	11.0	67	10.1	0.604
Irritated Throat	124	9.2	63	9.3	61	9.2	0.991
Irritated Eyes	120	8.9	63	9.3	57	8.6	0.689
Muscle Pain	105	7.8	55	8.1	50	7.5	0.729
Irritated Nose	99	7.4	61	9.0	38	5.7	**0.025**
Sore Throat	80	6.0	31	4.6	49	7.4	**0.027**
Cough	75	5.6	25	3.7	50	7.5	**0.002**
Heavy Breathing	70	5.2	34	5.0	36	5.4	0.707
Running Nose	68	5.1	31	4.6	37	5.6	0.381
Itching of Skin	39	2.9	21	3.1	18	2.7	0.695
Nausea	38	2.8	22	3.2	16	2.4	0.372
Blurred Vision	31	2.3	13	1.9	18	2.7	0.320
Feeling Faint	27	2.0	12	1.8	15	2.3	0.507
Abdominal Pain	25	1.9	8	1.2	17	2.6	0.058
Ephiphora	24	1.8	10	1.5	14	2.1	0.369
Diarrhea	21	1.6	9	1.3	12	1.8	0.465
Eye Injection	20	1.5	9	1.3	11	1.7	0.604
Eczema	19	1.4	7	1.0	12	1.8	0.222
Chest Tightness	16	1.2	6	0.9	10	1.5	0.286
Palpitation	16	1.2	5	0.7	11	1.7	0.116
Injuries	13	1.0	4	0.6	9	1.4	0.147
Taste Change	8	0.6	4	0.6	4	0.6	0.966
Vomiting	3	0.2	1	0.1	2	0.3	0.546

***P-value from chi-squared test or Fisher's exact test.**

Bold numbers indicate statistically significant results. (P<0.05)

Appendix Table 4.2: Demographic Factors by Background Occupation*

Demographic Factors	Citizen Volunteer		Company Employee		Military Personnel		P-value**
	N	%	N	%	N	%	
Number of Workers	189	100.0	402	100.0	743	100.0	
Age Median (Q1-Q3)	42.0 (34.0-49.0)		37.0 (29.0-43.8)		22.0 (21.0-25.0)		**<0.001**
Sex							
Male	151	79.9	351	87.3	742	99.9	**<0.001**
Female	38	20.1	51	12.7	1	0.1	

*9 workers with missing background occupation data

**P-Value from Kruskal-Wallis test for age and Fisher's exact test for sex.

Bold numbers indicate statistically significant results. (P<0.05)

Appendix Table 4.3: Adjusted Odds Ratio of Post-Shift Symptoms (per 1 pmol/ml increase in 1-OHPG) (n=1,318)*

Post-Shift Symptoms	Crude Odds Ratio**		Adjusted Odd Ratio***	
	OR	(95%CI)	OR	(95%CI)
Dizziness	0.94	(0.85 - 1.01)	0.93	(0.83 - 1.01)
Irritated Throat	**1.04**	**(1.00 - 1.08)**	**1.05**	**(1.01 - 1.10)**
Irritated Eyes	1.02	(0.97 - 1.06)	1.03	(0.98 - 1.07)
Muscle Pain	1.00	(0.92 - 1.04)	1.01	(0.95 - 1.06)
Irritated Nose	**1.04**	**(1.00 - 1.08)**	**1.05**	**(1.01 - 1.10)**
Sore Throat	0.96	(0.85 - 1.04)	1.00	(0.90 - 1.05)
Cough	**0.86**	**(0.71 - 0.99)**	**0.80**	**(0.64 - 0.94)**
Heavy Breathing	0.99	(0.88 - 1.05)	1.00	(0.88 - 1.06)
Running Nose	1.00	(0.91 - 1.05)	1.03	(0.96 - 1.08)
Itching of the Skin	1.02	(0.93 - 1.07)	1.02	(0.94 - 1.07)
Nausea	0.91	(0.72 - 1.04)	0.95	(0.75 - 1.06)
Blurred Vision	1.00	(0.86 - 1.07)	1.00	(0.85 - 1.06)
Feeling Faint	1.01	(0.87 - 1.07)	1.03	(0.88 - 1.10)
Abdominal Pain	0.96	(0.74 - 1.06)	0.97	(0.73 - 1.06)
Excessive Eye Tearing (Epiphora)	1.03	(0.93 - 1.08)	1.04	(0.94 - 1.09)
Diarrhea	**0.40**	**(0.16 - 0.76)**	**0.47**	**(0.18 - 0.91)**
Eye Injection (Redness)	1.05	(0.98 - 1.10)	1.05	(0.98 - 1.11)
Eczema	1.00	(0.80 - 1.07)	0.99	(0.77 - 1.06)
Chest Tightness	0.77	(0.43 - 1.03)	**0.64**	**(0.33 - 0.99)**
Palpitation	1.01	(0.83 - 1.08)	1.02	(0.82 - 1.09)
Injuries	0.76	(0.39 - 1.04)	0.79	(0.39 - 1.06)
Taste Change	0.95	(0.55 - 1.09)	0.98	(0.57 - 1.12)
Vomiting	Prevalence is too small to perform regression			

* 25 workers with missing symptom data

** Unadjusted odds ratio (Univariable model)

*** Odds ratio adjusted by urinary cotinine and days of cleanup (Multivariable Model)

Bold numbers indicate statistically significant results. (P<0.05)

Appendix Table 4.4: Adjusted Odds Ratio of Post-Shift Symptoms, Further Adjusting for Age of Workers (per 1 pmol/ml increase in 1-OHPG) (n=1,318)*

Post-Shift Symptoms	Crude Odds Ratio**		Adjusted Odd Ratio***	
	OR	(95%CI)	OR	(95%CI)
Dizziness	0.94	(0.85 - 1.01)	0.93	(0.83 - 1.01)
Irritated Throat	**1.04**	**(1.00 - 1.08)**	**1.04**	(1.00 - 1.09)
Irritated Eyes	1.02	(0.97 - 1.06)	1.02	(0.97 - 1.06)
Muscle Pain	1.00	(0.92 - 1.04)	1.01	(0.94 - 1.05)
Irritated Nose	**1.04**	**(1.00 - 1.08)**	**1.04**	**(1.00 - 1.09)**
Sore Throat	0.96	(0.85 - 1.04)	1.00	(0.90 - 1.05)
Cough	**0.86**	**(0.71 - 0.99)**	0.96	(0.79 - 1.05)
Heavy Breathing	0.99	(0.88 - 1.05)	0.99	(0.88 - 1.05)
Running Nose	1.00	(0.91 - 1.05)	1.03	(0.96 - 1.08)
Itching of the Skin	1.02	(0.93 - 1.07)	1.02	(0.93 - 1.07)
Nausea	0.91	(0.72 - 1.04)	0.94	(0.74 - 1.05)
Blurred Vision	1.00	(0.86 - 1.07)	0.98	(0.83 - 1.05)
Feeling Faint	1.01	(0.87 - 1.07)	1.05	(0.89 - 1.12)
Abdominal Pain	0.96	(0.74 - 1.06)	0.98	(0.74 - 1.07)
Excessive Eye Tearing (Epiphora)	1.03	(0.93 - 1.08)	1.03	(0.93 - 1.08)
Diarrhea	**0.40**	**(0.16 - 0.76)**	**0.46**	**(0.18 - 0.90)**
Eye Injection (Redness)	1.05	(0.98 - 1.10)	1.04	(0.96 - 1.09)
Eczema	1.00	(0.80 - 1.07	0.99	(0.78 - 1.06)
Chest Tightness	0.77	(0.43 - 1.03)	**0.63**	**(0.32 - 0.98)**
Palpitation	1.01	(0.83 - 1.08)	1.02	(0.82 - 1.09)
Injuries	0.76	(0.39 - 1.04)	0.80	(0.39 - 1.08)
Taste Change	0.95	(0.55 - 1.09)	0.98	(0.57 - 1.12)
Vomiting	Prevalence is too small to perform regression			

* 25 workers with missing symptom data

** Unadjusted odds ratio (Univariable model)

*** Odds ratio adjusted by urinary cotinine, days of cleanup and age of workers (Multivariable Model)

Bold numbers indicate statistically significant results. (P<0.05)

Appendix Table 4.5: Prevalence of Post-Shift Symptoms by Detectable t,t-MA

Post-Shift Symptoms	Total		Non-detectable		Detectable		P-value
	N	%	N	%	N	%	
Total Workers	1,343	100.0	907	100.0	436	48.1	
Dizziness	142	10.6	95	10.5	47	5.2	0.662
Irritated Throat	124	9.2	81	8.9	43	4.7	0.432
Irritated Eyes	120	8.9	86	9.5	34	3.7	0.382
Muscle Pain	105	7.8	67	7.4	38	4.2	0.157
Irritated Nose	99	7.4	72	7.9	27	3.0	0.746
Sore Throat	80	6.0	58	6.4	22	2.4	0.415
Cough	75	5.6	50	5.5	25	2.8	0.806
Heavy Breathing	70	5.2	44	4.9	26	2.9	0.328
Running Nose	68	5.1	52	5.7	16	1.8	0.149
Itching of the Skin	39	2.9	29	3.2	10	1.1	0.461
Nausea	38	2.8	28	3.1	10	1.1	0.893
Blurred Vision	31	2.3	19	2.1	12	1.3	0.528
Feeling Faint	27	2.0	18	2.0	9	1.0	0.775
Abdominal Pain	25	1.9	14	1.5	11	1.2	0.592
Excessive Eye Tearing (Epiphora)	24	1.8	15	1.7	9	1.0	0.676
Diarrhea	21	1.6	14	1.5	7	0.8	0.508
Eye Injection (Redness)	20	1.5	13	1.4	7	0.8	0.849
Eczema	19	1.4	12	1.3	7	0.8	0.920
Chest Tightness	16	1.2	12	1.3	4	0.4	0.187
Palpitation	16	1.2	12	1.3	4	0.4	0.499
Injuries	13	1.0	11	1.2	2	0.2	0.137
Taste Change	8	0.6	5	0.6	3	0.3	0.560
Vomiting	3	0.2	3	0.3	0	0.0	0.662

*P-value from chi-squared test or Fisher's exact test.

Appendix Table 4.6: Adjusted Odds Ratio of Post-Shift Symptoms by Detectable t,t-MA (n=1,318)*

Post-Shift Symptoms	Crude Odds Ratio**		Adjusted OR***	
	OR	(95%CI)	OR	(95%CI)
Dizziness	1.02	(0.70 - 1.47)	1.09	(0.73 - 1.62)
Irritated Throat	**1.04**	**(1.00 - 1.08)**	1.19	(0.77 - 1.82)
Irritated Eyes	0.80	(0.52 - 1.20)	0.82	(0.52 - 1.27)
Muscle Pain	1.19	(0.78 - 1.79)	1.40	(0.87 - 2.23)
Irritated Nose	0.76	(0.47 - 1.19)	0.92	(0.56 - 1.49)
Sore Throat	0.77	(0.46 - 1.26)	0.79	(0.44 - 1.38)
Cough	1.03	(0.62 - 1.68)	0.93	(0.50 - 1.68)
Heavy Breathing	1.23	(0.74 - 2.02)	1.31	(0.75 - 2.24)
Running Nose	0.62	(0.34 - 1.08)	0.62	(0.31 - 1.16)
Itching of the Skin	0.70	(0.32 - 1.41)	0.74	(0.31 - 1.61)
Nausea	0.73	(0.33 - 1.47)	0.95	(0.42 - 2.00)
Blurred Vision	1.31	(0.61 - 2.70)	1.30	(0.56 - 2.88)
Feeling Faint	1.03	(0.44 - 2.26)	0.88	(0.34 - 2.10)
Abdominal Pain	1.64	(0.72 - 3.63)	1.30	(0.49 - 3.31)
Excessive Eye Tearing (Epiphora)	1.24	(0.52 - 2.82)	1.22	(0.46 - 3.04)
Diarrhea	1.03	(0.39 - 2.50)	1.43	(0.47 - 4.01)
Eye Injection (Redness)	1.11	(0.42 - 2.74)	1.10	(0.37 - 3.00)
Eczema	1.21	(0.45 - 3.03)	1.06	(0.34 - 3.04)
Chest Tightness	0.69	(0.19 - 1.98)	0.42	(0.10 - 1.42)
Palpitation	0.68	(0.79 - 1.98)	0.65	(0.46 - 2.09)
Injuries	0.37	(0.06 - 1.39)	0.28	(0.04 - 1.27)
Taste Change	1.24	(0.25 - 5.08)	1.58	(0.29 - 7.08)
Vomiting	Prevalence is too small to perform regression			

* 25 workers with missing symptom data

** Unadjusted odds ratio (Univariable model)

*** Odds ratio adjusted by urinary cotinine and days of cleanup (Multivariable Model)

Bold numbers indicate statistically significant results. (P<0.05)

Appendix Table 4.7: Adjusted Odds Ratio of Post-Shift Symptoms by Detectable t,t-MA, Further Adjusting for Age of Workers (n=1,318)*

Post-Shift Symptoms	Crude Odds Ratio**		Adjusted OR***	
	OR	(95%CI)	OR	(95%CI)
Dizziness	1.02	(0.70 - 1.47)	1.11	(0.74 - 1.65)
Irritated Throat	**1.04**	**(1.00 - 1.08)**	**1.04**	**(1.00 - 1.09)**
Irritated Eyes	0.80	(0.52 - 1.20)	0.80	(0.50 - 1.24)
Muscle Pain	1.19	(0.78 - 1.79)	1.37	(0.85 - 2.19)
Irritated Nose	0.76	(0.47 - 1.19)	0.89	(0.53 - 1.44)
Sore Throat	0.77	(0.46 - 1.26)	0.77	(0.42 - 1.36)
Cough	1.03	(0.62 - 1.68)	0.95	(0.51 - 1.72)
Heavy Breathing	1.23	(0.74 - 2.02)	1.30	(0.74 - 2.22)
Running Nose	0.62	(0.34 - 1.08)	0.62	(0.31 - 1.17)
Itching of the Skin	0.70	(0.32 - 1.41)	0.70	(0.29 - 1.55)
Nausea	0.73	(0.33 - 1.47)	0.96	(0.42 - 2.03)
Blurred Vision	1.31	(0.61 - 2.70)	1.21	(0.51 - 2.70)
Feeling Faint	1.03	(0.44 - 2.26)	0.92	(0.36 - 2.19)
Abdominal Pain	1.64	(0.72 - 3.63)	1.34	(0.51 - 3.42)
Excessive Eye Tearing (Epiphora)	1.24	(0.52 - 2.82)	1.12	(0.42 - 2.83)
Diarrhea	1.03	(0.39 - 2.50)	1.41	(0.46 - 3.97)
Eye Injection (Redness)	1.11	(0.42 - 2.74)	0.95	(0.31 - 2.64)
Eczema	1.21	(0.45 - 3.03)	1.07	(0.34 - 3.08)
Chest Tightness	0.69	(0.19 - 1.98)	0.40	(0.10 - 1.36)
Palpitation	0.68	(0.79 - 1.98)	0.66	(0.16 - 2.11)
Injuries	0.37	(0.06 - 1.39)	0.30	(0.04 - 1.33)
Taste Change	1.24	(0.25 - 5.08)	1.59	(0.29 - 7.16)
Vomiting	Prevalence is too small to perform regression			

* 25 workers with missing symptom data

** Unadjusted odds ratio (Univariable model)

*** Odds ratio adjusted by urinary cotinine, days of cleanup and age of workers (Multivariable Model)

Bold numbers indicate statistically significant results. (P<0.05)

Appendix Table 4.8: Demographic Factors by Job Descriptions

Demographic Factors	Support Personnel		Contaminated Sand/Trash Removal		Oil Vacuum/ Oil Slick Removal		P-value*
	N	%	N	%	N	%	
Total Number	60	100.0	775	100.0	315	100.0	
Age Median (Q1-Q3)	44.5 (29.8-47.3)		24.0 (21.0-37.0)		25.0 (22.0-37.2)		**<0.001**
Sex							
Male	47	78.3	726	93.7	307	97.5	**<0.001**
Female	13	21.7	49	6.3	8	2.5	

*P-Value from Kruskal-Wallis test for age and Fisher's exact test for sex.

Bold numbers indicate statistically significant results. (P<0.05)

Appendix Table 4.9: Unadjusted Odds Ratio of Post-Shift Symptoms by Job Description (n=1,150)

Post-shift Symptoms	Support Personnel*	Contaminated Sand/ Trash Removal		Oil Vacuum/ Oil Slick Removal	
	OR	OR**	(95% CI)	OR**	(95% CI)
Dizziness	1.00 (Ref)	0.78	(0.38 - 1.82)	0.73	(0.33 - 1.79)
Irritated Throat	1.00 (Ref)	**0.27**	**(0.14 - 0.52)**	**0.20**	**(0.10 - 0.43)**
Irritated Eyes	1.00 (Ref)	0.57	(0.26 - 1.42)	0.98	(0.44 - 2.50)
Muscle Pain	1.00 (Ref)	1.71	(0.61 - 7.15)	1.71	(0.58 - 7.34)
Irritated Nose	1.00 (Ref)	**0.31**	**(0.16 - 0.67)**	**0.35**	**(0.16 - 0.79)**
Sore Throat	1.00 (Ref)	**0.48**	**(0.22 - 1.20)**	0.54	(0.23 - 1.43)
Cough	1.00 (Ref)	**NA**			
Heavy Breathing	1.00 (Ref)	**0.34**	**(0.16 - 0.83)**	**0.28**	**(0.11 - 0.74)**
Running Nose	1.00 (Ref)	2.04	(0.61 - 12.68)	1.14	(0.30 - 7.50)
Itching of the Skin	1.00 (Ref)	0.43	(0.14 - 1.86)	0.75	(0.23 - 3.38)
Nausea	1.00 (Ref)	**0.21**	**(0.08 - 0.61)**	0.29	(0.10 - 0.90)
Blurred Vision	1.00 (Ref)	0.46	(0.12 - 2.98)	0.85	(0.21 - 5.69)
Feeling Faint	1.00 (Ref)	**NA**			

*Coordinators, PTTGC Corporate Representatives, Visitors, Photographers, and Journalists were grouped as support personnel.

****Odds ratio from univariable model.**

Bold numbers indicate statistically significant results. (P<0.05)

NA because the prevalence of symptoms in the reference group is too small to calculate the odd ratio: 0/60 in cough, 0/60 feeling faint

For abdominal pain, epiphora, diarrhea, eye injection, eczema, chest tightness, palpitation, injuries, taste change and vomiting, the number of cases is too small to calculate accurate odds ratio.

Appendix Table 4.10: Adjusted Odds Ratio of Post-Shift Symptoms by Job Descriptions, Further Adjusting for Age of Workers (n=1,150)

Post-shift Symptoms	Support Personnel*	Contaminated Sand/Trash Removal		Oil Vacuum/ Oil Slick Removal	
	OR	OR**	(95% CI)	OR**	(95% CI)
Dizziness	1.00 (Ref)	0.98	(0.46-2.36)	0.92	(0.40-2.31)
Irritated Throat	1.00 (Ref)	**0.29**	**(0.14-0.59)**	**0.24**	**(0.11-0.55)**
Irritated Eyes	1.00 (Ref)	0.87	(0.38-2.25)	1.55	(0.65-4.15)
Muscle Pain	1.00 (Ref)	2.03	(0.67-8.82)	2.47	(0.77-11.12)
Irritated Nose	1.00 (Ref)	**0.43**	**(0.20-0.96)**	0.47	(0.20-1.13)
Sore Throat	1.00 (Ref)	**0.35**	**(0.14-0.97)**	0.53	(0.20-1.52)
Cough	1.00 (Ref)	**NA**			
Heavy Breathing	1.00 (Ref)	**0.34**	**(0.15-0.87)**	**0.0**	**(0.11-0.84)**
Running Nose	1.00 (Ref)	1.02	(0.27-6.71)	0.82	(0.20-5.64)
Itching of the Skin	1.00 (Ref)	0.38	(0.11-1.82)	0.67	(0.18-3.29)
Nausea	1.00 (Ref)	0.33	(0.12-1.02)	0.46	(0.15-1.53)
Blurred Vision	1.00 (Ref)	0.73	(0.17-5.08)	1.40	(0.30-10.23)
Feeling Faint	1.00 (Ref)	**NA**			

*Coordinators, PTTGC Corporate Representatives, Visitors, Photographers, and Journalists were grouped as support personnel.

****Odds ratio adjusted by urinary cotinine, days of cleanup and age of workers**

Bold numbers indicate statistically significant results. ($P<0.05$)

NA because the prevalence of symptoms in the reference group is too small to calculate the odds ratio: 0/60 in cough, 0/60 feeling faint

For abdominal pain, epiphora, diarrhea, eye injection, eczema, chest tightness, palpitation, injuries, taste change and vomiting, the number of cases is too small to calculate accurate odds ratio.

Appendix Table 4.11: Unadjusted Odds Ratio of Symptom Groups by Job Descriptions from Simple Logistic Regression and Factor Analysis (n=1,150)

Symptom Group	Support Personnel	Contaminated Sand/ Trash Removal		Oil Vacuum/ Oil Slick Removal	
	OR*	OR*	(95% CI)	OR*	(95% CI)
Chest Symptoms	1.00 (Ref)	NA		NA	
Allergic Skin Reaction	1.00 (Ref)	0.45	(0.15 - 1.97)	1.02	(0.32 - 4.47)
Irritative Symptoms	1.00 (Ref)	**0.45**	**(0.26 - 0.82)**	0.54	(0.30 - 1.01)
Gastrointestinal Symptoms	1.00 (Ref)	1.72	(0.35 - 31.14)	1.93	(0.36 - 35.83)
Respiratory Symptoms	1.00 (Ref)	1.01	(0.49 - 2.35)	0.86	(0.40 - 2.09)
Sensation Errors	1.00 (Ref)	1.32	(0.26 - 24.05)	2.34	(0.45 - 42.97)
Heavy Breathing	1.00 (Ref)	**0.34**	**(0.16 - 0.83)**	**0.28**	**(0.11 - 0.74)**

***Odds ratio from Univariable Model**

NA, sample size too small to calculate Odds Ratio. 0/60 support personnel had chest symptoms.

Bold numbers indicate statistically significant results. (P<0.05)

Appendix Table 4.12: Distributions of Post-Shift Symptoms by Background Occupation (n =1,318)**

Post-Shift Symptoms	Citizen Volunteer		Company Employee		Military Personnel		P-value*
	N	%	N	%	N	%	
Number of Workers	189	100.0	402	100.0	703	100.0	
Dizziness	29	15.3	39	9.7	74	10.5	**0.045**
Irritated Throat	35	18.5	30	7.5	59	8.4	**<0.001**
Irritated Eyes	32	16.9	23	5.7	65	9.2	**<0.001**
Muscle Pain	14	7.4	35	8.7	56	8.0	0.773
Irritated nose	31	16.4	29	7.2	39	5.5	**<0.001**
Sore Throat	14	7.4	16	4.0	50	7.1	0.104
Cough	3	1.6	6	1.5	66	9.4	**<0.001**
Heavy Breathing	19	10.1	10	2.5	41	5.8	**<0.001**
Running Nose	6	3.2	14	3.5	48	6.8	**0.046**
Skin Itching	9	4.8	13	3.2	17	2.4	0.146
Nausea	11	5.8	13	3.2	14	2.0	**0.009**
Blurred Vision	10	5.3	4	1.0	17	2.4	**0.004**
Feeling Faint	2	1.1	5	1.2	20	2.8	0.162
Abdominal Pain	1	0.5	4	1.0	20	2.8	**0.048**
Epiphora (Tearing)	3	1.6	8	2.0	13	1.8	0.944
Diarrhea	1	0.5	4	1.0	16	2.3	0.160
Eye Injection (Red)	7	3.7	4	1.0	9	1.3	**0.019**
Eczema	4	2.1	5	1.2	10	1.4	0.634
Chest Tightness	2	1.1	4	1.0	10	1.4	0.868
Palpitation	2	1.1	1	0.2	13	1.8	0.085
Injuries	1	0.5	1	0.2	11	1.6	0.107
Taste Change	3	1.6	0	0.0	5	0.7	**0.043**
Vomiting	0	0.0	1	0.2	2	0.3	1.000

***P-value from Chi-squared or Fisher's exact test**

** 25 workers with missing symptom data

Bold numbers indicate statistically significant results. (P<0.05)

Appendix Table 4.13: Adjusted Odds Ratio of Post-Shift Symptoms by Background Occupation (n=1,318) **

Post-Shift Symptoms	Citizen Volunteer	Company Employee		Military Personnel	
	OR	OR*	95%CI	OR*	95%CI
Dizziness	1.00 (Ref)	0.60	(0.36-1.03)	0.78	(0.47-1.30)
Irritated Throat	1.00 (Ref)	**0.37**	**(0.21-0.63)**	**0.23**	**(0.14-0.40)**
Irritated Eyes	1.00 (Ref)	**0.31**	**(0.17-0.55)**	**0.58**	**(0.35-0.96)**
Muscle Pain	1.00 (Ref)	1.18	(0.61-2.38)	0.45	(0.22-0.92)
Irritated nose	1.00 (Ref)	**0.36**	**(0.21-0.62)**	**0.27**	**(0.15-0.49)**
Sore Throat	1.00 (Ref)	0.50	(0.23-1.09)	**0.37**	**(0.18-0.77)**
Cough	1.00 (Ref)	1.02	(0.26-4.95)	1.92	(0.63-8.35)
Heavy Breathing	1.00 (Ref)	**0.23**	**(0.10-0.50)**	0.56	(0.30-1.07)
Running Nose	1.00 (Ref)	0.99	(0.38-2.87)	0.72	(0.27-2.15)
Itching of the Skin	1.00 (Ref)	0.52	(0.21-1.36)	**0.20**	**(0.07-0.53)**
Nausea	1.00 (Ref)	0.54	(0.23-1.26)	**0.32**	**(0.13-0.80)**
Blurred Vision	1.00 (Ref)	**0.18**	**(0.05-0.56)**	**0.35**	**(0.14-0.88)**
Feeling Faint	1.00 (Ref)	1.33	(0.28-9.44)	2.10	(0.54-14.0)

***Odds ratio adjusted by urinary cotinine and days of cleanup**

**25 workers with missing symptom data

Bold numbers indicate statistically significant results. (P<0.05)

For abdominal pain, epiphora, diarrhea, eye injection, eczema, chest tightness, palpitation, injuries, taste change and vomiting, the number of cases is too small to calculate accurate odds ratio.

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Theses

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